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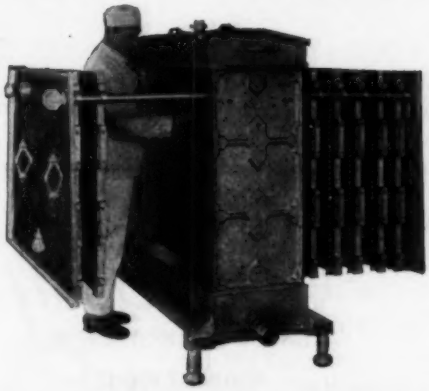

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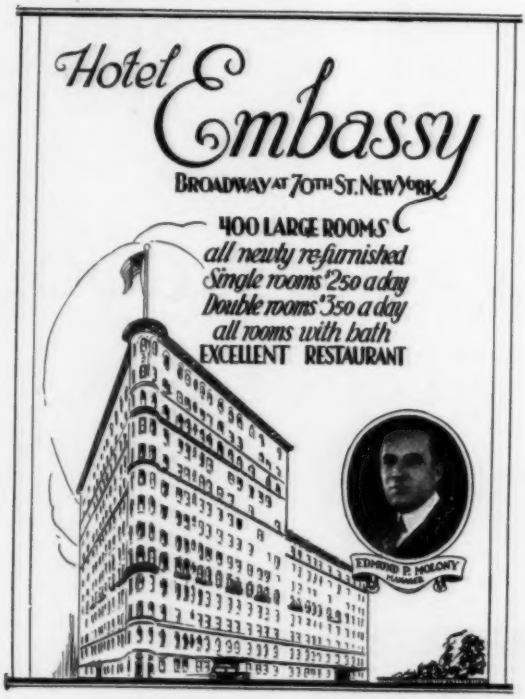

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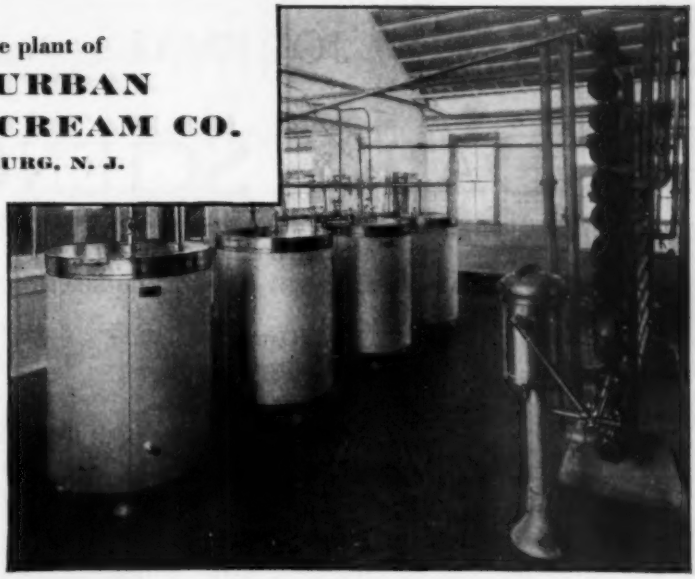

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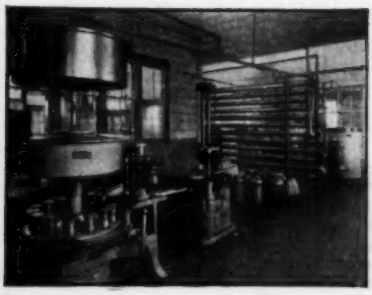

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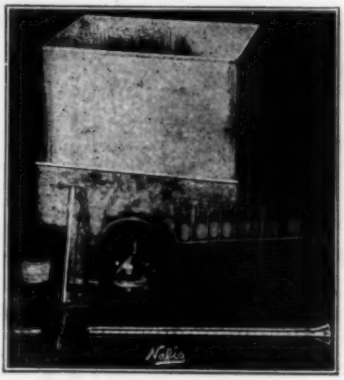

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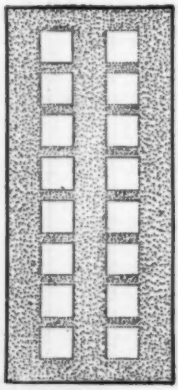

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SIGNIFICANCE OF COLON-AEROGENES GROUP IN ICE CREAM*

I. SURVIVAL OF MEMBERS OF THE ESCHERICHIA-AEROBACTER GROUP TO PASTEURIZING TEMPERATURES IN ICE CREAM

F. W. FABIAN AND E. W. COULTER

Michigan State College, East Lansing, Michigan

INTRODUCTION

The sanitary significance of particular groups of bacteria in food products is of peculiar interest. This is especially true with the Escherichia-Aerobacter group for several reasons. In case of ice cream considerable importance is attached by some to the presence of this group. In this connection several questions present themselves. What are the possibilities of this group gaining entrance into ice cream? Does the presence of this group in retail ice cream indicate inefficient pasteurization or contamination after pasteurization? Is the health officer justified in condemning ice cream which contains members of the Escherichia-Aerobacter group? What is the possibility of members of this group surviving the pasteurizing temperature now in use?

It was to gain specific information on the last named question and general information on some of the other questions raised that this work was undertaken.

The scientific literature contains numerous papers on the bacteriology of ice cream, but very few of these have special reference to the Escherichia-Aerobacter group of organisms. Buchan (3) in 1910 made a complete bacteriological study of 40 small ice cream plants in England. Out of 66 cultures isolated and identified 47 belonged to the Escherichia-Aerobacter group. Isolation was accomplished by the use of bile salt lactose agar. The

* Received for publication December 23, 1929. Published by permission of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 28 (N. S.). Data from thesis presented in partial fulfillment of the requirements for the Master of Science degree in Michigan State College by the junior author.

author presented a bacteriological standard for ice cream which limited members of the Escherichia-Aerobacter group to 0.1 cc. of ice cream as measured by the production of acid and gas in bile salt glucose broth. One of the more recent papers on this subject is that of Weinzirl and Harris (16). Three methods for determining the sanitary quality of ice cream were studied and compared. These were the total count, the Escherichia-Aerobacter count and the anaerobic spore test. The authors concluded that the Escherichia-Aerobacter count is of value in controlling the efficiency of pasteurization and that it also indicates insanitary conditions. The fact that it does not distinguish between initial contamination and subsequent multiplication was cited as a distinct disadvantage.

This brief summary indicates the need of further contributions to this field of research. The greatest need seems to be for a standard method of interpreting the significance of members of this group when found in ice cream. However, it is evident that this problem when applied to ice cream presents difficulties not present when applied to milk. The numerous differences between these two dairy products demand more investigation before definite conclusions may be reached. Very little work has been done to indicate those factors which may or may not affect the viability of members of this group in ice cream. This phase alone should be of sufficient value to attract further attention.

PROCEDURE

To test the ability of the Escherichia-Aerobacter group to survive pasteurizing temperatures in ice cream 44 cultures were subjected to three different temperatures. Three cultures of *Aerobacter aerogenes* were isolated from water, 2 from soil, and 2 laboratory stock cultures were used. A total of 33 cultures of *Escherichia coli* were tested. Of this number 13 were isolated from water, 11 were of avian origin, 3 of bovine, 1 of equine, 2 of human, 1 of porcine, 1 of monkey and 1 a laboratory stock culture of unknown origin. In addition to these, 4 lactose-fermenting organisms isolated from water but not definitely classified were also used.

Dolloff's synthetic medium, (6) standard agar and lactose broth to which had been added 1 per cent Andrade's indicator were used. The ice cream mix used in the pasteurizing experiments contained 12 per cent butter fat, 14 per cent sucrose, and 37 per cent total solids. The mix was obtained directly from the holding tank and had been pasteurized previously at 62.8°C. (145°F.). Ten cubic centimeters of this mix were then distributed in pyrex test tubes and heated for 30 minutes in flowing steam for two successive days. The tubes were tested for sterility by seeding into lactose broth.

An experiment was carried out according to the following procedure. The culture to be tested was grown in Dolloff's medium for 24 hours at 37°C. Ten cubic centimeters of sterile ice cream mix were then inoculated with 0.1 cc. of this liquid culture which contained from ten thousand to fifty thousand bacteria as determined by the plate method. The culture was thoroughly stirred into the mix after which the tube was incubated for 2 hours at 37°C. At the end of this time one loop-full (4 mm.) of the material always produced abundant growth and gas in lactose-broth at the end of 24 hours when incubated at 37°C.

At the end of the 2 hours incubation period the inoculated tubes of ice cream mix were placed in an electrically heated and controlled De Khotinsky type water bath which permitted the accurate regulation of the bath temperature to within 0.1°C. After adjusting the bath to the desired temperature, the tube was immersed to within ½ inch of the top. A similar tube of ice cream mix containing a calibrated thermometer was placed along side of the seeded tube. The 30 minute period of heating which was used for all the tests was not recorded until the temperature of the material in the tube containing the thermometer had reached the desired temperature. The temperatures used were 60°C. (140°F.), 62.8°C. (145°F.), 65.6°C. (150°F.), 68.3°C. (155°F.).

Immediately after the 30 minute heating period the tubes were immersed in cold water and 0.1 cc. of the heated mix seeded into a lactose broth fermentation tube. This was incubated at 37°C. for 48 hours and growth recorded. Any fermentation tube showing 5 per cent gas and sufficient acid to change Andrade's indica-

tor at the end of this period was recorded as positive, indicating a survival of the culture at this temperature. Our tests showed that while this method was a more delicate means of indicating the survival of the bacteria to the different degrees of heat used

TABLE 1

The effect on viability of heating members of the Escherichia-Aerobacter group in ice cream mix and in skim milk at a temperature of 60°C. (140°F.) for 30 minutes. Result of four trials

CULTURE NUMBER	ICE CREAM TRIAL NUMBER				SKIM MILK TRIAL NUMBER				CULTURE NUMBER	ICE CREAM TRIAL NUMBER				SKIM MILK TRIAL NUMBER			
	1	2	3	4	1	2	3	4		1	2	3	4	1	2	3	4
2*W	+	−	+	+	−	−	−	−	54A	−	+	−	+	+	+	−	+
3**W	°	°	−	−	−	−	−	−	55A	+	−	−	−	+	−	−	+
7W	°	°	−	−	−	−	−	−	56A	−	−	−	+	+	−	−	+
14*W	°	°	−	−	−	−	−	−	57A	+	−	−	−	−	−	−	−
18W	−	+	+	+	+	+	−	+	58A	−	+	−	+	+	−	−	+
19W	+	+	+	+	−	−	−	−	59A	+	+	−	+	−	−	−	+
29*X	+	−	−	−	−	−	−	−	60A	+	+	−	+	−	−	−	+
30X	−	−	+	+	−	−	−	−	100W	+	−	−	+	−	−	−	+
31*X	−	−	+	+	−	−	−	−	101W	+	+	−	+	−	+	−	+
34**W	−	−	−	−	−	−	−	−	103W	+	+	−	+	+	+	−	+
35**W	−	−	−	−	−	−	−	−	104B	+	+	−	+	+	−	+	+
37**W	−	−	−	−	−	−	−	−	105B	−	+	−	−	−	−	−	−
38W	+	+	+	+	−	+	+	+	107A	−	−	°	−	−	−	−	−
39W	+	+	−	+	−	−	+	−	108E	−	+	°	+	+	−	−	+
40W	+	−	+	−	−	−	−	−	109W	+	−	°	−	+	−	+	−
45W	−	−	+	+	−	−	−	−	110M	−	−	°	−	−	−	−	−
46*W	−	−	+	+	−	−	−	−	112H	−	−	°	−	−	−	−	−
47W	−	−	−	+	−	−	−	+	113H	+	−	°	+	+	+	−	+
49A	+	+	−	+	−	+	+	−	114P	+	−	°	−	−	−	−	−
50A	+	−	−	−	−	−	−	−	116B	−	+	°	+	+	−	+	+
51A	−	−	−	−	−	−	+	−	120*S	+	−	°	+	+	+	−	+
53A	−	+	−	+	+	−	−	+	121*S	+	−	°	−	−	−	−	−

(+) = growth; (−) = no growth; (°) = not determined; (**) = unidentified; (*) = Aerobacter aerogenes; all others *Escherichia coli*. W = water; X = origin unknown; A = avian; B = bovine; E = equine; M = monkey; H = human; P = porcine; S = soil.

than the plate method, yet there was sufficient variation to render the results questionable without several trials. In view of this fact, the entire mix in the tube was incubated at 37° for 48 hours and held at room temperature for 48 hours longer and re-

tested as a check on the lactose fermentation tube method. A negative test was recorded, therefore, only when the lactose fermentation tube failed to show positive and no bacteria were demonstrable in the original mix which had been heated in the tube. This procedure was adopted in view of the work of Ayers and Johnson (2) on "majority" and "absolute" thermal death points of bacteria in milk.

The first temperature used was 60°C. In this experiment the cultures were tested in sterile skim milk as well as in sterile ice cream for comparison. In view of previous work which had

TABLE 2

Showing variability of different cultures when heated at 62.8°C. (145°F.) for 30 minutes in ice cream. Results of four trials

CULTURE NUMBER	NUMBER OF HEATING			
	1	2	3	4
18	+	+	−	+
38	+	+	−	−
39	+	+	−	−
40	+	+	−	−
49	+	−	+	−
60	+	+	+	−
103	−	+	−	−
104	+	+	+	−
107	+	+	−	−
113	+	+	−	−

shown considerable variation when the same cultures were heated several times at the same temperature, the cultures were heated 4 times under identical conditions to test their degree of variability. The results are given in detail in table 1.

On the first trial of the 41 cultures tested, 21 or 51.2 per cent of them survived. Sixteen or 39.0 per cent out of the 41 cultures survived the second trial, while in the third trial only 25 cultures were tested 9, or 36.0 per cent of which survived and on the fourth trial 25, or 57.0 per cent out of the 44 cultures survived.

There were two cultures which appeared to be very resistant to heating in ice cream since they survived all the heatings. Nine

cultures survived 3 heatings in ice cream while the rest were variable. Eleven of the cultures were very sensitive to heat and were killed in each instance.

Skim milk presents quite a different picture. Twenty-two cultures failed to survive any of the heatings. There were none that survived all the heatings; 8 which survived 3 trials while the rest were variable. These data indicate the protective action of ice cream.

TABLE 3

Recapitulation of results secured by heating at temperatures of 60°C., 62.8°C. and 65.5°C. members of the Escherichia-Aerobacter group four times in ice cream

TRIAL NUMBER	TOTAL NUMBER OF CULTURES HEATED	NUMBER KILLED	NUMBER SURVIVING	PER CENT SURVIVING
60°C. (140°F.) for 30 minutes				
1	41	20	21	51.2
2	41	25	16	39.0
3	25	16	9	36.0
4	44	19	25	57.0
62.8°C. (145°F.) for 30 minutes				
1	44	34	10	22.7
2	44	41	3	6.8
3	44	43	1	2.2
4	44	34	10	22.7
65.5°C. (150°F.) for 30 minutes				
1	40	40	0	0
2	41	40	1	2.4
3	41	40	1	2.4
4	44	40	4	9.1

The two most resistant of the cultures were *Escherichia coli* isolated from water. While the number of cultures of *Aerobacter aerogenes* as compared to *Escherichia coli* is too small to draw definite conclusions, yet it would appear from these data that there was little difference in the heat resistance of the two groups.

The temperature was then raised to 62.8°C. for 30 minutes and the experiment repeated with the same cultures using ice

cream and skim milk. Not a single culture survived any of the 4 heatings at this temperature in skim milk. The results were different in ice cream, however, as shown in table 2. In this table only those cultures surviving at this temperature are listed, all the others having been killed. The results of this experiment are given in table 2.

A study of this table reveals that with one exception (culture 107) the cultures surviving at 62.8°C. were likewise resistant to 60°C. However, the number surviving was greatly reduced at the higher temperature since only 10 showed growth at 62.8°C. while 33 survived one or more heatings at 60°C.

Considerable variation in the resistance to heat of different cultures is likewise exhibited in the different trials. In the first trial all survived except one while in the fourth trial all were killed except one.

Since a fairly large percentage of the cultures survived at temperature of 62.8°C. for 30 minutes in ice cream, the temperature was increased to 65.5°C. and the experiment repeated. The results of this experiment are given in table 3. A summary of the results obtained at 62.8°C. and 60°C. are also given for comparison.

Cultures number 18, 29, 38, 39 and 50 survived 65.5°C. Culture 38 survived 2 out of the 4 trials while all the rest survived only 1 out of the 4 trials.

The experiment was repeated again with ice cream. This time the temperature was raised to 68.3°C. and held for 30 minutes. All the cultures were killed.

INFLUENCE OF SEPARATE CONSTITUENTS OF ICE CREAM MIX UPON THERMAL DEATH POINT OF COLON-AEROGENES ORGANISMS

Since the results showed that ice cream mix had a greater protective action than skim milk, further experiments were carried out using the same procedure to determine the effect of each ingredient separately.

In conducting these experiments an attempt was made to study these materials in the same concentration in which they occur normally in the ice cream mix. As the formulae used by the ice cream manufacturers are varied according to the demands

of the consumers, each ingredient was made up in concentrations which would include those used in the various formulae available.

Emulsions of fat were made from 40 per cent cream as a base. In order to avoid changing the solids not fat content, weighed amounts of cream were added to weighed amounts of water. The percentages used were 8 (pH = 6.86), 10, 12, 14, and 16 (each having a pH of 6.94) per cent respectively of butter fat by weight. This material was distributed in 10 cc. amounts in Pyrex tubes and autoclaved for 15 minutes at 10 pounds pressure and checked for sterility. The rest of the procedure was the same as

TABLE 4

The effect on the viability of members of the Escherichia-Aerobacter group when heated in various water suspensions of skim milk powder at a temperature of 60°C. (140°F.) for 30 minutes

CULTURE NUMBER	1 PER CENT pH 6.86		2 PER CENT pH 6.86		4 PER CENT pH 6.70	
	Acid	Gas	Acid	Gas	Acid	Gas
50	−	−	−	−	−	−
51	−	−	−	−	−	−
53	−	−	−	−	−	−
54	−	−	−	−	−	−
55	−	−	−	−	+	+
56	+	+	−	−	−	−
57	−	−	−	−	−	−
58	+	+	−	−	−	−
59	+	+	−	−	−	−

that previously described for the ice cream mix. The temperature used was 60°C. for a period of 30 minutes. Since all the results were consistently negative, they have not been tabulated.

Skim milk powder. Water suspensions of it were prepared in 1, 2, and 4 per cent concentrations. Sterilization was accomplished by successive heatings in flowing steam. The 9 cultures used had previously survived a temperature of 60°C. in skim milk. The results are given in table 4.

The experiments were repeated at a temperature of 62.8°C. for 30 minutes with consistently negative results so they have not been tabulated.

Sucrose was made up in 10, 12 and 14 per cent solutions and tubed in 10 cc. quantities, sterilized at 10 pounds pressure for 15 minutes. The cultures used in this experiment were the same

TABLE 5

The effect on the viability of members of the Escherichia-Aerobacter group when heated in various concentrations of sucrose at a temperature of 60°C. (140°F.) for 30 minutes

CULTURE NUMBER	10 PER CENT pH 6.69		12 PER CENT pH 6.52		14 PER CENT pH 6.18	
	Acid	Gas	Acid	Gas	Acid	Gas
50	−	−	−	−	−	−
51	−	−	−	−	+	+
53	−	−	−	−	−	−
54	−	−	−	−	+	+
55	+	+	−	−	−	−
56	−	−	−	−	−	−
57	−	−	−	−	−	−
58	+	−	+	−	+	−
59	−	−	−	−	−	−

TABLE 6

The effect of the age of the culture when members of the Escherichia-Aerobacter group are heated in ice cream mix at a temperature of 61.6°C. (143°F.) for 30 minutes

NUMBER	6 HOURS		12 HOURS		24 HOURS	
	Acid	Gas	Acid	Gas	Acid	Gas
18	−	−	−	−	+	+
19	−	−	−	−	+	+
38	−	−	−	−	+	+
39	+	−	+	−	−	−
40	+	−	+	−	+	+
60	+	−	+	−	+	+
101	+	−	−	−	+	+
103	−	−	−	−	−	−
Con	−	−	−	−	−	−

as used in skim milk. Three or 33 per cent survived a temperature of 60°C. for 30 minutes. However, all were killed when the temperature was raised to 62.8°C. and held for 30 minutes. The results at 60°C. are given in table 5.

It will be noted that sucrose shows a slight protective action.

Gelatin was tested from two sources of supply. Emulsions were made in concentrations ranging from 0.2 to 1.0 per cent at 0.10 per cent intervals. The pH of each concentration from each supply was determined and found to vary quite widely. The pH of 0.5 per cent solutions of the samples tested were 5.00, 5.67, 5.84, and 7.12 respectively. Nine of the more resistant strains were heated at 62.8°C. for 30 minutes in these different concentrations. All the tests were consistently negative. For this reason the data have not been presented in tabulated form. The acidity of most of the gelatin tested would preclude the possibility of any of the organisms surviving.

THE EFFECT OF AGE OF THE CULTURE

Eight of the cultures showing the greatest resistance to heat were grown for 6, 12 and 24 hours and at the end of these periods were heated to 61.6°C. (143°F.) for 30 minutes. This temperature was chosen since it was found to be the temperature at which the majority of the above strains were still viable at the end of 30 minutes. The results are given in table 6.

DISCUSSION

The data submitted have provided some interesting facts which should prove useful in interpreting the significance of members of the Escherichia-Aerobacter group in ice cream. The comparison of the effect on viability produced by heating the different cultures in skim milk and in ice cream at the various temperatures for 30 minutes indicated definitely that the latter exhibited a greater protective action.

The testing of the ingredients contributed very little to a solution of the problem as a whole. Of all the substances tested fat was the one from which the greatest protective action was expected. The results of heating of the cultures in fat suspensions showed great variability. However, the organisms were readily killed when heated at a temperature of 60°C. for 30 minutes. The data show no influence of concentration of fat upon viability.

Heating the cultures in skim milk powder showed no correla-

tion between concentration and viability. The cultures surviving a temperature of 60°C. when heated in skim milk powder were the same cultures surviving this temperature when heated in skim milk. Also three of these cultures survived in ice cream mix at the same temperature.

Sucrose showed some protective action since some of the cultures survived at 60°C.

Gelatin was the one ingredient tested which exhibited the least protective effect at the concentration used (0.5 per cent). The lowest temperature adopted, 60°C., was sufficient to kill all cultures heated.

The marked effect exhibited by the age of culture was found to be in accord with previous work of this nature. Thus Robertson (11) found that young cells were more susceptible than older cells to the action of high temperatures. He concluded that the most efficient pasteurization would be obtained if the heat was applied before the bacteria had passed the accelerative stage of growth. Sherman and Albus (12) found that young cells of bacteria are more easily killed by heat and various harmful agents then are the old cells of the same organism. Sherman, Stark and Stark (13) more recently have reviewed the literature in this connection and have given additional data to support this view.

The variations and apparent inconsistencies were not entirely unexpected since other workers have found similar variations. Ayers and Johnson (1) studied the effect on viability of 174 cultures of *Escherichia coli* when heated in milk and found the following variations: At a temperature of 62.8°C., 12 cultures survived the first trial. On retesting they found 4 cultures surviving the second trial, 8 surviving the third trial, 6 surviving the fourth trial, 9 surviving the fifth trial, and no cultures surviving the sixth trial. The litmus milk tube method was employed. The authors concluded that a temperature of 65.5°C. for 30 minutes should destroy members of the Escherichia-Aerobacter group when heated in milk. Tanner and Windsor (15) studied 23 cultures of *Escherichia coli* and found only one strain that repeatedly withstood heating in sealed tubes for 30 minutes at 62.8°C. If it is common for milk to show marked variations, it would not be surprising that the ice cream mix should exhibit even greater variations.

The question of pH undoubtedly is closely linked with viability. According to Cohen (5) mortality is affected by small changes in pH. This factor would be impossible to control except under laboratory conditions. The mix when stored in the holding tank slowly undergoes a decrease in pH. Thus freshly made mix was found to have a pH of 6.8, whereas after 48 hours in the holding tank the pH had often dropped to 5.5. The determination of the significance of this factor would constitute a separate problem in itself.

One of the outstanding characteristics of ice cream mix is its viscosity. It seems reasonable to assume that this property might have some influence on the protective action exhibited by the mix. Extensive work by Joslyn (8) on sirups showed the relationship between viscosity and heat penetration. He found that an increase in viscosity resulted in a decrease in the rate of heat penetration and that the addition of acid reduces the retardation of heat penetration. This factor which has little significance under laboratory conditions, might be of importance in the commercial pasteurization process.

The fact that ice cream mix does possess considerable protective action for members of the Escherichia-Aerobacter group is significant. Thus it is reasonable to assume that this protection might be accorded to other groups of bacteria. If this assumption is correct, it is evident that a temperature of 62.8°C. cannot effectively pasteurize ice cream mix.

SIGNIFICANCE OF ESCHERICHIA-AEROBACTER IN ICE CREAM

Inasmuch as ice cream is a dairy product and the conditions under which dairy products are produced precluded the possibility of entirely eliminating members of this group, it would seem that their presence in ice cream is to be expected to a certain extent. Furthermore, it has been shown that certain strains are able to survive the temperature which is generally used in pasteurizing ice cream mix viz. 62.8°C. for 30 minutes. It would seem, therefore, that qualitative tests would be of little value in this connection. Definite quantitative tests plus a history of the sample being tested would be of value.

It has been pointed out by Mudge (9) and Price (10) that the significance of the colon organism when found in milk is in no way as great as when found in water.

There are some, however, who consider that *Escherichia coli* is a valuable index of pasteurizing efficiency and that it can be used to check up on plant performance. Jenkins (7) in England studied the flora of pasteurized milk and stated that an effectively pasteurized milk should not contain lactose fermenting bacilli in one cc. and that *Escherichia coli* is a valuable index of the efficiency of the pasteurizing process. Swenarton (14) likewise believes that a test for *Escherichia coli* content can be used to check up on plant procedure since the charts indicated a definite correlation between coli content and plant procedure. He found that control charts from milk plants whose milk was high in coli content showed improper heating or irregularity of procedure.

The one other factor of interest, namely, that of contamination after pasteurization is one which should be given due consideration. Probably the most common source of contamination of this type is from the hands of operators. The possibility of this source of *Escherichia coli* was recognized by Winslow (17) as early as 1903. A more recent article by Buice, Sehested and Dienst (4) reported 337 tests made on hands of 251 food handlers. They found 67.7 per cent of the total number showing lactose fermenting aerobes. By culturing with Koser's sodium citrate medium, 8.4 per cent of the total proved to be of intestinal origin. Close attention to personal hygiene would eliminate this factor as one of importance.

SUMMARY AND CONCLUSIONS

1. The thermal death point of 33 cultures of *Escherichia coli*, 7 cultures of *Aerobacter aerogenes* and 4 lactose-fermenting organisms isolated from water but not definitely classified showed considerable variation when heated in ice cream at temperatures of 60°C. (140°F.), 62.8°C. (145°F.) and 65.5°C. (150°F.) for 30 minutes.

Four determinations were made at a temperature of 60°C. The number surviving expressed in per cent was 51.2, 39.0, 36.0

and 57.0 respectively for each determination. When the temperature was increased to 62.8°C., the number surviving decreased somewhat being 22.7, 6.8, 2.2 and 22.7 per cent respectively for the successive determinations. At a temperature of 65.5°C. the number surviving is still further decreased and was 0.0, 2.4, 2.4 and 9.1 per cent respectively while at a temperature of 68.3°C. none of the cultures tested survived.

These data indicate that the critical temperature for the Escherichia-Aerogenes group in ice cream is about 65.5°C.

2. Ice cream has a greater protective action than skim milk for members of the Escherichia-Aerogenes group when heated at temperatures of 60°C. and 62.8°C. for 30 minutes. At the former temperature in skim milk there were 22 cultures out of the 44 cultures used which failed to survive any of the four heatings as compared to 11 cultures in ice cream. When the temperature was raised to 62.8°C. all the cultures were killed in skim milk in each instance while in ice cream there were certain cultures still surviving.

3. Thermal death point determinations made with the cultures in the different ingredients used in making ice cream, viz. cream, sucrose, milk powder and gelatin failed to show any marked protective action of any one of the ingredients.

4. The susceptibility of bacterial cells to heat is greatly influenced by their age, young cells are more readily killed than are the older ones.

5. The ability of many strains of the Escherichia-Aerogenes group to survive a temperature of 62.8°C. and even 65.5°C. in ice cream should be taken into consideration when using the colon test as an index of the efficiency of pasteurization.

6. From the standpoint of reduction in numbers of the Escherichia-Aerogenes group, pasteurizing the ice cream mix at a temperature of 65.5°C. is more desirable than the lower temperature 62.8°C. which is more commonly used.

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A CASE OF SANDINESS IN PROCESSED CHEESE*

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This brief article is to record the occurrence of "sandiness" in processed cheese. Two samples of sandy processed cheese were examined independently by the writer and by the Wisconsin Dairy and Food Commission chemists, respectively. In the former case, the sample was submitted by the manufacturer for identification of the gritty particles and diagnosis of the defect; in the latter case, the sample was obtained from a retailer after a consumer had complained that the cheese contained broken glass. It is not definitely known, but it is likely that both samples originated from the same source.

The gritty particles were easily visible to the naked eye and were obviously crystalline. The appearance and sensation on chewing was as though quartz sand had been incorporated into the cheese. Figure 1 is a photomicrograph, magnification 24 times, of the gritty particles removed from the cheese.

Some of the gritty particles were removed with the aid of a scalpel, and cleaned by removing adhering cheese and washing successively with water, alcohol and ether. The crystals were soluble in hydrochloric and nitric acids, and disintegrated in sulphuric acid, leaving a fine precipitate (calcium sulphate). Calcium was demonstrated to be present by the oxalate test applied either to a solution obtained by dissolving the residue after ashing some of the particles, or to a solution obtained by dissolving some of the particles in nitric acid, evaporating to dryness and taking up the residue with water. The acid in combination with calcium was found to be an organic acid. The crystal structure of the gritty particles suggested that they consisted of calcium tartrate. Tartaric acid was demonstrated by the re-

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ducing action on silver nitrate in ammoniacal solution causing the deposition of a silver mirror in a clean test tube, and by Denige's modification of Mohler's Resorcinol Test. The conclusion was that the gritty particles were calcium tartrate. Both laboratories working independently and without knowledge of the other's work on a similar sample, arrived at the same conclusion. In the case of the sample submitted by the manufacturer it was defi-

FIG. 1. PHOTOMICROGRAPH OF CALCIUM TARTRATE CRYSTALS TAKEN FROM SANDY OR GRITTY PROCESSED CHEESE. DARK FIELD ILLUMINATION, MAGNIFICATION 24 ×

nitely ascertained that the processed cheese had been made with the addition of Rochelle Salts as an emulsifying agent.

In an isolated attempt to produce this sandy condition by processing cheese with the addition of 2.0 per cent Rochelle Salts and 0.2 per cent calcium acetate, the sandy condition did not result. The solubility of calcium tartrate is 0.016 parts by weight

per 100 parts of water at 15°C. From this fact, from the known water and calcium content of average cheese, and from the fact that emulsifying agents in processed cheese manufacture are used in amounts ranging from 0.5 to 3.0 per cent, it is quite certain that precipitation of calcium tartrate always occurs in processed cheese when tartrate is used as the so-called emulsifying agent. The question of whether or not the cheese becomes sandy or gritty must then be a question of the size of calcium tartrate crystals, rather than their presence or absence. A number of conditions likely to affect the size of the calcium tartrate crystals suggest themselves, but have not been subjected to experimental study.

CONCLUSION

In two samples of gritty or sandy processed cheese, the cause of the defect was the presence of calcium tartrate crystals of macroscopic size.

OPTIMUM AMOUNT OF SILAGE IN THE DAIRY RATION FOR MOST ECONOMICAL PRODUCTION*

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PRELIMINARY STATEMENT

Following the introduction of the silo on farms in the eighties a conviction of the great importance of corn silage in the dairy ration has become almost universal. These opinions have been formed and sustained by numerous reports from experiment stations and by practical feeders as well. In more recent years the ensiled product of various other plants has been reported favorably. Moreover the extension of the uses of silage to beef cattle production and to the feeding of sheep and horses has been acclaimed with enthusiasm.

The point of view with which the subject usually has been approached experimentally in dairy cattle feeding has involved an attempt to discover the influence upon milk yield of substituting silage for a portion of the dry roughage of the ration. Under such trials the animals have usually responded by an increased yield and this has furnished the basis for proclaiming silage as the one feed to be desired above all others, without adaptation to any particular farm, area, or region.

During the past few years the Farm Management Department of the Connecticut Agricultural College has observed the cost of corn production in Connecticut to be relatively high compared to hay production, due to the intensive labor and fertilizer requirements of corn. Furthermore, this matter has attracted the attention of the farmers themselves, with the result that many have turned to large Southern varieties in an effort to reduce the acreage devoted to corn and to obtain cheaper silage. In more

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recent years corn production for silage has been curtailed to a noticeable extent, and in a few cases abandoned, even on large farms. This situation applies at least in southern New England and probably constitutes a similar problem in other sections as well.

In conjunction with other considerations in the silage feeding trials under way at Storrs since 1921, succeeding variety tests by the Department of Agronomy, the question of the optimum amount of silage for dairy cattle rations under prevailing conditions has continually injected itself into the problem. Only recently has it been possible to attack the problem directly. The preceding trials have successively shown: (*a*) that large Southern varieties[1] which yield the largest gross tonnage of dry matter are capable of producing the most milk per acre of corn (1); (*b*) that although such varieties yield silage containing considerably less grain and a lower percentage of dry matter, accompanied by a somewhat larger percentage of fiber per unit of dry matter, yet the dry matter of such corns does not suffer appreciably in feeding value, the result being that the acre dry matter production is a better criterion for measuring the production ability of a given variety of corn than its ability to form ears in an average growing season (2); (*c*) a cow receiving the standard amount of silage will adjust her total dry matter consumption by ingestion of hay in amount to correspond with the dry matter content of the silage (unpublished data (3)).

In consideration of the change in economic conditions favoring more and better hay production on tillable land, and with an appreciation of the marked advance that has taken place in the feeding of better grain rations to cows during the past few years, the authors felt that it was timely to attack the problem from this new point of view. The question may be propounded: "Does the cow now respond as favorably to silage as she did upon the prevailing rations in use at the time of and for several years following the introduction of the silo?"

[1] The growing season for corn is about 120 days in Connecticut.

HISTORICAL STATEMENT

It does not seem appropriate to review here all of the numerous experiments in which silage has had an important rôle. Only those involving considerations of corn silage and hay which afford sufficient data to permit of a comparison of the total dry matter consumed, the resulting milk yield, and feed costs of the milk, will be mentioned.

Most of the early investigators were concerned directly in finding whether silage could be substituted for hay, without much attention to the proportion of each which would prove most efficient and economical. Due to the usual greater yield of dry matter from an acre of corn, few, until recent years, seem to have considered that corn silage may on some farms and in some areas be more costly than hay.

Hills (4) in 1889, Bartlett (5) in 1889, and Wheeler (7) in 1895 all report greater milk yield and more efficient use of dry matter when corn silage replaced a part of the hay in the ration.

Fairchild and Wilbur (8) at Indiana, Carroll (9) at Utah, and the Arizona Station (10) report an increase in milk production by replacement of a part of the alfalfa hay by silage. Silage is estimated to have a value ranging from 28 to 40 per cent of that of an equal weight of alfalfa, although in two cases no analyses are given.

The results referred to, together with the data of the present trial, are summarized in table 7. Wherever possible the yields have been calculated to 4 per cent milk using the formula of Gaines and Davidson (6) ($0.4 \times$ milk $+ 15 \times$ fat).

OBJECT OF EXPERIMENT

In actual feeding practice upon the dairy farm the relative amounts of corn silage and home grown hay available determine to a large extent the proportions of each in the ration. Under conditions prevailing in Connecticut (limited tillable land on many farms) faith in silage often dictates the production of corn on tillable areas to the exclusion of hay except on permanent hay fields, necessitating the use of much poor hay and the limited feeding thereof.

Studies in recent years by the Farm Management Department at the Connecticut Agricultural College have revealed that Connecticut farmers expend about 80 hours of man labor in growing and ensiling an acre of corn, and that a ton of silage costs the Connecticut dairyman fully half as much to produce and harvest as a ton of hay.

These findings together with results in silage investigations by the Department of Dairy Husbandry, already referred to, have raised the pertinent question as to what proportion of hay and silage in the ration is desirable in the interest of greatest profits.

The experiments referred to uniformly indicate that corn silage when added to a ration tends to increase milk flow. Many practical feeders, although not all, will testify to this fact. Notwithstanding the somewhat overwhelming evidence in this direction some important questions yet remain unanswered, namely: (*a*) what is the optimum amount of silage for a dairy cow necessary to maintain satisfactory yield; (*b*) what is the minimum amount of silage capable of furnishing succulence to the ration; (*c*) what is the feed cost of milk production using varying proportions of silage in the ration and hay ad libitum in each case; (*d*) may not the improved quality of hay and of grain mixtures have lessened the favorable influence of silage as determined by earlier feeders and investigators?

It is hoped that the present and subsequent experiments will throw light on such questions. This experiment (the eighth in silage investigations) was designed to show whether light or heavy silage in the ration would be, *first*, more efficient and *second*, more economical.

EXPERIMENTAL PROCEDURE

Eight purebred cows, 4 Holsteins and 4 Guernseys, were divided into two groups evenly balanced with respect to breed, body weight, age, milk production, and stage of lactation. The animals were also closely balanced with respect to their body type and ability to consume roughage, factors of much importance in an experiment of this kind.

Table 1 gives data concerning the experimental cows.

The groups, it will be observed, are especially well balanced, perhaps excepting date of conception. At this stage of gestation it can be assumed that there was little influence upon results due to this factor.

One group was fed 36 pounds of silage and the other 18 pounds per cow. The grain was fed in proportion to milk flow and hay allowed ad libitum in two feedings.

Two preliminary periods of 10 days each (January 2–21, inclusive) were provided to allow each animal to be changed over

TABLE 1

Data concerning the animals

NAME	BREED	AGE	CALVED	CONCEIVED	INITIAL WEIGHT
Heavy Silage group					
Delight	Holstein	2- 9-14	8-31-28	11-21-28	1,270
Senora	Holstein	2-10-20	8- 9-28	11-18-28	1,185
Amiable	Guernsey	4- 1-16	10-15-28	12-25-28	1,002
Sagacious	Guernsey	5- 4-13	12- 8-28	1-18-29	1,050
Average age and weight		3- 9-16			1,127
Average days from calving	89				
Light Silage group					
Korndyke	Holstein	2- 8-15	8-12-28	1-26-29	1,162
Queen	Holstein	2-10-19	8- 5-28	11-20-28	1,278
Buoyant	Guernsey	4- 9-12	12-20-28	2-10-29	1,054
Laureate	Guernsey	5- 8-18	11- 6-28	1- 1-29	1,123
Average age and weight		4- 0- 8			1,154
Average days from calving	91				

from herd routine, to become accustomed to the ration and stabilized in weight and production. The experiment proper in addition consisted of ten 10-day periods from January 22 to May 1 inclusive.

Since the roughages and the grain fed to both groups were from the same source daily there was no appreciable opportunity for variation in quality of roughage or grain to affect the experimental results.

Silage feeding

The silage was of the variety *early mastodon*, classed as moderately late maturing, and containing a small proportion of developed ears. Analysis revealed an average dry matter content of 21.90 per cent. The silage was removed from the silo daily with that fed the main herd and was weighed into the manger from the feed truck morning and evening. All silage refused was weighed and recorded. The silage was sampled at the middle of each 10-day period and air dried immediately with the aid of a fan before submitting for analysis.[2]

Hay feeding

The hay was fed twice daily, immediately following the consumption of silage and grain, in such amounts as the individual cows preferred. Any portion not eaten by an animal was weighed and recorded.

Since it was planned to make the results of this experiment applicable to average Connecticut herds it seemed advisable to use average hay. It was decided that mixed hay containing some clover should be used. Because of the extremely rainy season of 1928 and the difficulty experienced in curing, the hay, which was purchased from a Connecticut farm, did not prove to be of very good quality. It was cut fairly early and contained not over 15 per cent clover, but many bales were musty, making careful sorting necessary. A characteristic flake was removed from each bale and composited for the 10-day sample. Later a composite was removed from each 10-day sample for an experimental sample. The sample covering the first half of the experimental period was inadvertently destroyed (by an employee) so the analyses given for hay cover only the last half of the period. Observation indicated that the sample as analyzed probably was somewhat superior to that fed during the first half of the period.

[2] Acknowledgment is due to the Department of Analytical Chemistry of the State Station at New Haven for the analyses of all feeds.

Grain feeding

The grain fed was a stock mixed open formula ration with a guarantee of 20 per cent protein prepared by the Eastern States Farmers' Exchange. The analysis, shown in table 3, was made from a composite sample from every fifth bag. The ingredient formula follows:

	pounds
Corn Gluten Feed	380
Choice Yellow Hominy	340
Linseed Oil Meal O. P.	320
Pure Ground Oats (No. 2—38 pounds clipped)	260
Standard Wheat Bran	200
Choice Cottonseed Meal	140
Corn Distillers Dried Grains	100
Standard Wheat Middlings	100
Molasses	100
Steamed Bone Meal	20
Calcium Carbonate	20
Salt	20

The grain was fed approximately at the ratio of 1 pound to each 3 pounds of milk produced, with allowance for differences in test, but was varied individually, however, in an attempt to hold body weight uniform. The grain was fed twice daily on the silage.

Management of cows

The cows were kept in stanchions and turned out an hour or two daily for exercise when the weather was suitable. Water was before them at the stanchions. Since the grain ration contained 1 per cent salt no additional salt was fed. Wooden manger partitions were installed to prevent the animals from thieving. Sawdust was used for bedding so there was no opportunity to consume straw and vitiate the dry matter intake. Each animal was weighed daily between 8 and 9 A.M. The weights as presented are an average of the weights the day before, the day of, and the day following the date given in table 5. The cows were fed silage and grain at 5 A.M. and at 4 P.M. After these were eaten hay was fed. The cows were hand milked while consuming silage and grain.

Presentation of data

There was a tendency on the part of 6 of the 8 cows to consume more hay during the preliminary period than they could continue

TABLE 2

Group feed records

	HEAVY SILAGE GROUP			LIGHT SILAGE GROUP		
	Silage	Hay	Grain	Silage	Hay	Grain
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
First preliminary period....	1,434.3	455.6	380.00	720	578.1	377.6
Second preliminary period..	1,438.5	447.5	361.8	720	535.1	361.8
First experimental period...	1,440.0	440.3	336.1	720	527.1	342.3
Second experimental period.	1,411.4	436.2	316.1	720	539.4	323.8
Third experimental period..	1,431.5	374.3	309.1	720	540.8	311.5
Fourth experimental period.	1,440.0	365.4	301.1	720	512.6	301.8
Fifth experimental period...	1,438.4	361.4	293.5	720	512.1	282.6
Sixth experimental period...	1,440.0	371.6	296.0	720	471.1	275.5
Seventh experimental period.	1,436.5	370.1	292.7	720	484.5	254.1
Eighth experimental period.	1,428.0	350.3	287.3	720	473.5	267.9
Ninth experimental period..	1,468.5	337.9	286.0	720	475.6	279.1
Tenth experimental period..	1,473.9	341.5	276.0	720	483.0	277.5
Total (100 days).....	14,408.2	3,749.0	2,993.9	7,200.0	5,019.7	2,916.1
Average per day, experimental period.....	36.02	9.37	7.48	18.00	12.55	7.29

TABLE 3

Average analyses of feeds in per cent

	DRY MATTER	MOISTURE	ASH	PROTEIN	FIBER	N-FREE EXTRACT	FAT
Silage.....	21.90	78.10	1.42	1.73	6.60	11.59	0.56
Hay.....	92.84	7.16	5.83	8.25	36.24	40.85	1.67
Grain.....	91.35	8.65	6.50	21.13	7.71	51.55	4.46

to eat, evidence of which is found in the records of feed consumption by 10-day periods in table 2.

It will be noted also that the grain feeding was unnecessarily high at the start, especially for the lower producers, necessitating rather sharp reductions during the first few periods.

TABLE 4

Group production records

	HEAVY SILAGE GROUP			LIGHT SILAGE GROUP		
	Milk	Fat	4 per cent milk	Milk	Fat	4 per cent milk
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
First preliminary period....	1,223.7	50.866	1,252.4	1,112.8	46.881	1,148.4
Second preliminary period..	1,174.4	47.130	1,174.8	1,038.7	48.977	1,150.0
First experimental period...	1,122.5	43.912	1,107.6	985.1	46.163	1,086.8
Second experimental period.	1,054.4	36.397	967.6	938.7	42.047	1,006.4
Third experimental period..	993.6	37.926	956.4	883.2	40.803	965.6
Fourth experimental period.	953.5	34.431	898.4	837.5	40.299	939.6
Fifth experimental period...	925.3	35.388	900.8	824.4	38.416	906.0
Sixth experimental period...	927.2	36.258	914.8	790.0	35.639	850.8
Seventh experimental period.	857.6	34.642	862.8	706.9	31.389	753.6
Eighth experimental period.	801.5	31.151	788.0	722.7	32.829	781.6
Ninth experimental period..	738.3	29.266	734.4	681.1	31.018	737.6
Tenth experimental period..	678.6	28.709	702.0	658.1	29.663	708.0
Total (100 days).....	9,052.5	348.080	8,832.8	8,027.7	368.266	8,736.0
Average per day, experimental period.....	22.63		22.08	20.07		21.84

TABLE 5

Average body weights of groups

	FIRST PRELIMINARY PERIOD		SECOND PRELIMINARY PERIOD		FIRST EXPERIMENTAL PERIOD		SECOND EXPERIMENTAL PERIOD	
Date.....	1–3	1–8	1–13	1–18	1–23	1–28	2–2	2–7
Heavy Silage group.....	1,135	1,135	1,137	1,139	1,140	1,140	1,133	1,129
Light Silage group.....	1,154	1,148	1,139	1,143	1,134	1,128	1,127	1,124
	THIRD EXPERIMENTAL PERIOD		FOURTH EXPERIMENTAL PERIOD		FIFTH EXPERIMENTAL PERIOD		SIXTH EXPERIMENTAL PERIOD	
Date.....	2–12	2–17	2–22	2–27	3–4	3–9	3–14	3–19
Heavy Silage group.....	1,120	1,114	1,103	1,105	1,105	1,103	1,110	1,114
Light Silage group.....	1,127	1,130	1,121	1,121	1,113	1,108	1,114	1,101
	SEVENTH EXPERIMENTAL PERIOD		EIGHTH EXPERIMENTAL PERIOD		NINTH EXPERIMENTAL PERIOD		TENTH EXPERIMENTAL PERIOD	
Date.....	3–24	3–29	4–3	4–8	4–13	4–18	4–23	4–28
Heavy Silage group.....	1,113	1,118	1,117	1,117	1,109	1,118	1,118	1,119
Light Silage group.....	1,086	1,097	1,092	1,085	1,087	1,094	1,093	1,090

TABLE 6

Group consumption of dry matter in pounds

PERIOD	HEAVY SILAGE GROUP				LIGHT SILAGE GROUP			
	Silage	Hay	Grain	Total	Silage	Hay	Grain	Total
First preliminary	333.47	422.98	347.13	**1,103.58**	167.40	536.71	344.94	**1,049.05**
Second preliminary	349.99	415.46	330.50	**1,095.95**	175.18	496.79	330.50	**1,002.47**
First experimental	325.44	408.77	307.03	**1,041.24**	162.72	489.36	312.69	**964.77**
Second experimental	306.98	404.97	288.76	**1,000.71**	156.60	500.78	295.79	**953.17**
Third experimental	314.93	347.50	282.36	**944.79**	158.40	502.08	284.56	**945.04**
Fourth experimental	338.83	339.24	275.05	**953.12**	169.42	475.90	275.69	**921.01**
Fifth experimental	322.63	335.52	268.11	**926.26**	161.50	475.43	258.16	**895.09**
Sixth experimental	325.15	344.99	270.40	**940.54**	162.58	437.47	251.67	**851.72**
Seventh experimental	312.01	343.60	267.38	**922.99**	156.38	449.81	232.12	**838.31**
Eighth experimental	305.74	325.22	262.45	**893.41**	154.15	439.60	244.73	**838.48**
Ninth experimental	310.29	313.71	261.26	**885.26**	152.14	441.55	254.96	**848.65**
Tenth experimental	292.57	317.05	252.13	**861.75**	142.92	448.42	253.50	**844.84**
Total experimental (100 days)	3,154.57	3,480.57	2,734.93	**9,370.07**	1,576.81	4,660.40	2,663.87	**8,901.08**

Table 3 presents the chemical analyses of feeds used during the experiment. The silage analyses are an average of the 10 samples for the experiment proper; however for purposes of computation the several individual analyses were used for their respective periods.

Table 4 gives the group production of milk and fat by periods, also the 4 per cent fat corrected milk. Gaines and Davidson (6) submit abundant data justifying the practice of correcting milk production to 4 per cent fat basis for purposes of comparison.

Average group weights are given in table 5.

The Heavy Silage group lost an average of 21 pounds per animal while the Light Silage group lost an average of 44 pounds from the first 3 days of the first experimental period to the last 3 days of the last experimental period.

From the analyses given for hay and grain, and from silage analyses for the respective periods, and also from the data on food consumption in Table 2 the dry matter consumption for the two groups was computed. This is presented in table 6 for the several periods.

DISCUSSION

Reference to table 2 will show that both groups consumed more hay for the first two experimental periods than they were capable of consuming indefinitely at the level of silage intake. The break in hay consumption of the Light Silage group in the sixth experimental period was due to the fact that Queen went off feed. She was a cow with considerable total capacity for roughage consumption, similar in this respect to Delight in the other group, and ate more over a period of time than proved to be advantageous to herself. This parallels and compensates the action of Delight who was more than any other responsible for the decline in hay consumption of the Heavy Silage group during the third experimental period. These examples illustrate the need of a long experimental period and also illustrate the opportunity for a residual effect to be carried over into the experimental period if the reversal type of experiment is employed. It will be noticed that the Heavy Silage group continued to decline in hay con-

sumption to the ninth experimental period, influenced in part perceivably by advancing lactation.

It will be noted from table 5 that the two groups were evenly balanced in weight at the beginning of the preliminary period and had an approximately equal weight at the beginning of the experimental period proper. There are three changes in the weights which deserve explanation; first, the Light Silage group decreased in weight during the preliminary periods probably due to loss of fill of the intestinal tract; second, the loss in weight of the Heavy Silage group occurred in the third period when Delight over-ate followed by a decrease in grain consumption temporarily; and, third, decline in the weight of the Light Silage group which occurred March 19 was due to an udder injury to Korndyke causing a decline in milk production and necessitating a temporary cut in grain feeding. At this same time Queen, in the same group, over-ate, adding to the decline in weight.

An analysis of the production of 4 per cent milk and the consumption of grain for the two groups shows that the Heavy Silage group produced 2.951 pounds of milk and the Light Silage group 2.996 pounds of milk per pound of grain fed, or that the Light Silage group received slightly less grain per pound of milk produced. Since the two groups were carefully balanced in all respects and were fed almost an identical ratio of grain per pound of milk produced it is reasonable to assume that any differences in efficiency and economy of production are directly due to the relative proportions of the dry matter coming from silage and hay respectively. Reference to table 6 shows that the Light Silage group failed by 397.9 pounds of dry matter to eat sufficient additional hay to compensate for the lower silage allowance. This doubtless accounts for the greater loss of weight of this group, 44 pounds per animal against 21 for the other group.

With respect to milk production per pound of dry matter consumed the Light Silage group produced 0.98 of a pound of milk and the Heavy Silage group 0.94 of a pound of milk for each pound of dry matter in the ration. Expressing this in another way the Light Silage group consumed 102 pounds of dry matter to 106 pounds by the Heavy Silage group for each 100 pounds of

4 per cent milk yielded. This seemingly greater efficiency of the Light Silage group has the greater loss of body weight to be charged against it, which for want of an accurate correction factor must be assumed to balance the two groups. Both differ-

TABLE 7

Comparison of work at several stations showing the pounds of milk produced per pound of dry matter fed and the per cent of dry matter provided by the hay and silage*

STATION	PERIOD	4 PER CENT MILK PER POUND DRY MATTER	PER CENT DRY MATTER	
			Hay	Silage
Connecticut (Storrs)	Heavy Silage	0.94	37.1	33.7
	Light Silage	0.98	52.4	17.8
Maine	No Silage	0.94	73.6	
	Heavy Silage	0.90	50.6	26.0
	No Silage	0.86	74.8	
Vermont	No Silage	0.74	70.1	
	Silage	0.81	40.2	27.2
Geneva	No Silage	0.67	72.7	
	Light Silage	0.72	51.0	20.3
	Heavy Silage	0.77	27.6	41.2
Geneva	Heavy Silage	0.92	27.6	42.8
	Light Silage	0.91	46.1	24.0
Geneva	Light Silage	0.94	49.7	22.8
	Heavy Silage	1.00	26.0	45.2
Purdue	Heavy Silage	0.94	35.9	39.1
	No Silage	0.92	75.0	

* Computed by the authors to a 4 per cent fat basis.

ences, however, are well within the range of experimental error, or chance.

Table 7 presents a comparison of results of other experiments with these under discussion.

In evaluating the data in table 7 it should be kept in mind that the number of animals in each group is small, but it seems signif-

icant that all of the older data indicates more efficient production with the larger amounts of silage. Furthermore, silage feeding has resulted in a more efficient production in respect to the dry matter of the ration than when no silage was fed. But from the data in these experiments it is impossible to determine the changes in weight of the groups under comparison.

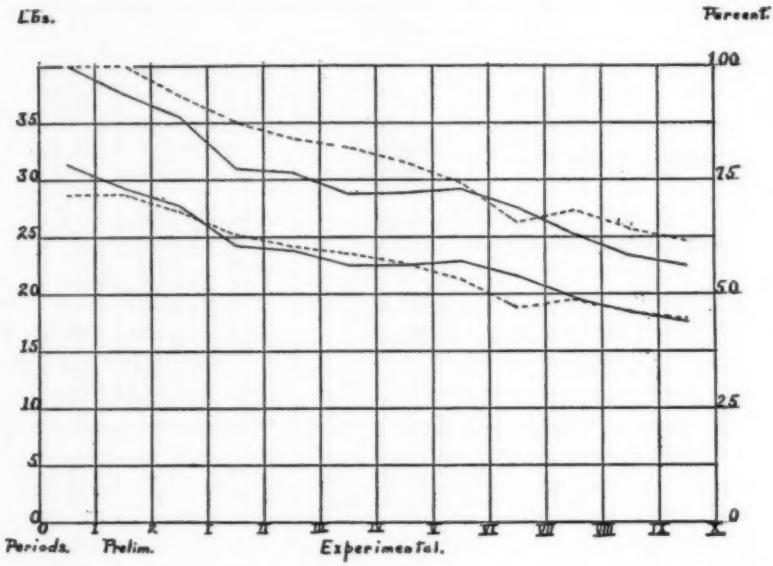

FIG. 1. DECLINE OF 4 PER CENT MILK

The upper graph is expressed as the percentage decline taking the first month as 100, while the lower graph is expressed in pounds.
Heavy Silage group ————.
Light Silage group ----------.

The Storrs data, which is in disagreement, may or may not be significant. The problem requires further experiment.

In studying all of the data it is found that there is after all comparatively little difference in efficiency of rations with large or small amounts of silage, and that the ultimate feeding practice will depend therefore upon whether or not silage in quantity

stimulates greater milk flow or appetite, and the relative cost of dry matter from silage and hay.

Turner (11) in studying the rate of decline of milk production found that on an average the monthly milk yield is 93.95 per cent of that of the preceding month. The Light Silage group averaged 95.4 per cent of the previous months production while the Heavy Silage group averaged 95.0 per cent. In this respect the groups seem to have been well balanced and the yield well maintained. Turner found that food consumption averaged 97.98 per cent of that of the previous month. The Light Silage group averaged 98.2 per cent while the Heavy Silage group averaged 97.7 per cent. Likewise the results were similar in this respect. Figure 1 illustrates the decline of milk production in pounds and in per cent.

The Farm Management Department at the Connecticut Agricultural College has estimated from farm accounts the cost of production of silage at $9.00 per ton and of hay at $16.00 per ton (unpublished data). With grain at cost ($52.35 laid down at the barn in the winter of 1928–29 wholesale) and roughage at the above prices, the feed cost of producing 100 pounds of 4 per cent milk was $1.95 for the Heavy Silage group and $1.70 for the Light Silage group. This difference in economy of production resulted in a 14 per cent greater income above feed cost for the Light Silage group despite the slightly greater milk yield of the Heavy Silage group.

By calculation it is found that the prices of hay and silage would have to be established at $25.00 and $5.00 respectively, a relation which would scarcely be possible, to make the feed cost approximately equal for the two groups. At these assumed prices for roughage the feed cost per 100 pounds of milk of the Heavy and Light Silage groups would be $1.82 and $1.80 respectively.

Adjusting the price of grain to correspond to different geographical areas will not materially alter the relationship. It would seem from this data that the hay is worth about five times as much as the silage. Wheeler (Geneva), from his experiments, stated that with hay at $10.00 per ton the silage was worth $2.62. At this ratio with hay valued at $16.00 per ton the silage would

have to be produced at $4.19 per ton to be on a par in cost. The silage in this instance (Wheeler's experiment) contained 26.34 per cent dry matter.

SUMMARY

Two groups of milk cows were placed on experiment for a preliminary period of 20 days and an experimental period of 100 days. One group received 36 pounds of corn silage daily while the other received but 18 pounds. Both were fed approximately 1 pound of grain for each 3 pounds of 4 per cent milk produced and mixed hay, largely timothy, ad libitum.

Heavy silage feeding resulted in slightly greater dry matter consumption and in slightly greater milk production. The Light Silage group produced slightly more milk per unit of dry matter, but on the other hand lost more weight. However the amount of weight lost in either case is no more than would be expected for cows in the first half of their lactation when producing efficiently.

With hay at $16.00, silage at $9.00, and grain at $52.35 per ton the feed cost of 100 pounds of milk was $1.95 for the Heavy Silage group and $1.70 for the Light Silage group. In this trial the income over feed cost was 14 per cent greater for the Light Silage group. With silage at $5.00, hay at $25.00 per ton, and grain the same ($52.35) the feed cost per 100 pounds of milk for the Heavy Silage group becomes $1.82 and that for the Light Silage group $1.80. A shift in the price of grain does not materially alter the spread in the cost between the total rations.

It is realized that these results will be received with much surprise and doubt by many advocates of heavy silage feeding. They should serve to stimulate the study of the adaptation of silage production to the conditions existing on a given farm and area. In so far as certain commercial interests are concerned, which in recent months have brought unscientific charges against silage in comparison with certain feed treatment processes, it is hoped that they will receive no impetus from these results in furtherance of their pernicious propaganda.

This experiment is being repeated this year under the same

conditions, essentially, so this paper may be regarded as a progress report.

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THLASPI ARVENSE (FRENCH WEED) IN RELATION TO DAIRY PRODUCTS*

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An especially obnoxious defect in cream and butter on several occasions has been brought to the attention of the authors. Investigation of conditions at one creamery showed several cream producers were delivering a product with the particular taste and odor in question. Indications pointed to the feed used as a source of the trouble. The grain ration contained a large proportion or grain screenings. The experiences of Canadian butter manufacturers and references in the literature, especially those of Canadian origin, suggested the possibility that the objectionable conditon might result from the seeds of the *Thlaspi arvense*, known under a variety of local names, but most commonly in Western Canada and in Minnesota as French weed. The presence of seeds of this specie in the screening feed used was demonstrated by the staff of the Minnesota Seed Control Laboratory. As the result of these observations, the experiments reported were initiated.

The literature on the subject is quite extensive, and numerous observations are reported, especially in Canadian publications, which indicate rather clearly the objectionable features of this plant. The plant is known by a variety of names including Penny cress in Europe; Mithradate mustard in Gray's Manual of Botany; Wild garlic; and Fanweed. In Manitoba and the Dakotas, it is generally called French weed or Stinkweed. It is a member of the mustard family.

The plant in appearance resembles the common Shepard's purse (*Bursa pastoris*), from which it is distinguished by the pods, which as a rule are about three-fourths of an inch across, flat, and circular, having a well defined wing around the margin and a

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notch at the apex. The plant is both an annual and a winter annual. Seed formation begins by May and continues on plants of different ages until freezing weather in the fall. When moistened, the seeds have a nauseous garlic-like taste and a pungent odor. The green plant shows these same characteristics but to a less degree.

Authorities agree that the weed is an immigrant from Europe, where it is said to be especially common in France. The presence of *Thlaspi arvense* seeds in rape seed cake from India, mentioned by Werenskiold (1), suggests that this pest may also be found in India. Apparently, it made its way to the North American continent some years ago. Darlington (2) states that it was known in Michigan from 1860 to 1881.

In 1892, Waldron (3) listed it as a troublesome weed that had recently appeared in North Dakota. The same year, McKay (4) reported French weed as one of the especially troublesome weeds that had recently attracted attention in various parts of Canada. He also reported special trouble from it in Manitoba. Reports on the same subject were published by Clark and Fletcher (5). Howitt (6) in 1908 mentions it as one of the worst pests of the grain fields and states that one means of dissemination is through the agency of ground screenings and as an impurity in farm seeds.

These and other writers including Shaw (7) emphasize particularly the injury to the small grain crops caused by this pest but in some cases also mentioned the plant as giving a bad taste to milk and to the beef of animals which feed upon it. Other authors mention that it is necessary in certain localities to keep cattle off pastures containing this plant for from two to three weeks previous to slaughter in order that the carcass may be free from the objectionable taste.

French weed taint is quite common in cream received at butter factories in Manitoba and Sasketchewan, according to information received by correspondence from the Dairy Commissioners and members of the staff of the Agricultural Colleges in these Provinces. In these regions, the trouble appears most commonly early in the spring and sometimes late in the fall, presumably

from cows' eating the weed in question when the pasture grass is not abundant. It also appears that sometimes trouble is caused from animals eating the chaff and waste where grain has been threshed. Cases of French weed tainted cream occasionally appears during the winter in these Provinces, probably from the cows' eating hay containing the dried plant.

The French weed has been known in the Northwestern part of Minnesota for probably thirty-five years, but only in recent years has it become a serious pest. In 1928, the State department of Agriculture issued posters and other information calling attention to the obnoxious character of the weed. It is found at present generally distributed in the Red River Valley and to a considerable extent to the east and south in Minnesota.

The fact that this weed grows chiefly with small grain results in the presence of the seeds in screenings. Immense quantities of screenings are available in Minnesota on account of the extensive milling industry. A considerable part of the screenings secured from grain grown in the prairie section of Canada also find their way into the hands of the commercial organizations interested in the preparation of mixed feed in the United States. According to the Dominion Department of Agriculture, screenings to the amount of about 100,000 tons taken from Canadian export grain were shipped from the Lake ports into the United States in 1927.

As a result of the facts brought to light concerning the danger to dairy products from French weed seed, some commercial feed mixers have recently installed elaborate machinery to remove the French weed and common mustard seed from the screenings. This reduces the danger from the use of commercial feeds containing screenings. Undoubtedly in earlier years especially, the lack of care in grinding screenings was a factor in the spread of French weed as well as of other obnoxious species.

EXPERIMENTAL

The reports in the literature regarding the injurious effects of French weed upon dairy products are based upon general observations rather than controlled experiments. In order to

obtain accurate information, a series of experiments were conducted for the purpose of studying: (*a*) the effect of various amounts of French weed seed upon the quality of milk and butter; (*b*) the relation of the length of interval from the time consumed to the appearance of the objectionable taste and odor; (*c*) the effect of feeding the green French weed plant; (*d*) the efficiency of common practices for removing the objectionable flavor and odor; (*e*) the possibility of detecting by easily applied methods the presence of French weed seed in mixed feeds.

The French weed seed used was obtained from a commercial feed and seed company which has in use machinery designed to remove the seed of this weed from grain screenings. The material as obtained was found by the seed analyst of the State Department of Agriculture to contain 22 per cent of French weed seed. By the use of various screens and a fanning mill, it was possible to concentrate the French weed portion until it was reported to represent 50 per cent of the total. A closer concentration seemed impractical without hand picking. The remainder was composed of the seeds of pigweed, foxtail, lambs quarter, barnyard grass, and Canada thistle. These seeds did not have a pronounced taste and were therefore ignored. In giving the weights of French weed used in the experiments, it should be understood that an allowance is made for the foreign seeds present. The seed mixture containing the French weed was thoroughly ground to remove the danger of seed distribution through the alimentary canal of the animals and to imitate the conditions in which the seed usually is found in commercial feeds.

The ground seed was fed mixed with the regular grain mixture used for the University herd. It will not be necessary to report the numerous experiments in detail. It was found that the animals ate the feed mixture containing the ground seed freely the first time, but when fed a second lot, the same animal often ate the mixture with evident lack of relish. In some cases, the feed was refused entirely. This result was experienced when no more than 150 grams of the French weed seed was added to 8 pounds of mixed feed. In a later series, the French weed seed was mixed with feeding molasses and then with the grain. By this proce-

dure, the animal would readily consume the prescribed amount. It was noted that a strong odor of garlic appeared almost as soon as the feed was moistened in the cow's mouth. By the time the eating of the grain was completed, the typical odor of French weed could be detected as much as ten feet from the feed box. Shortly after the feed was consumed, the breath of the cow began to show a pronounced French weed odor. The pungent odor was sufficiently strong from the breath to be distinctly irritating to a person's eyes when near.

The milk was drawn either by hand or with a machine. It was examined carefully when fresh and again after standing in a closed container for at least 12 hours. The milk was then separated. After examination, the cream was churned and the butter examined from time to time for a period of about a month. It was found that for a cow producing milk at the rate of 30 pounds daily, 90 grams of French weed fed two hours before milking did not produce any noticeable effect. The intake was then increased to 200 grams, which was found to give a decided effect upon the milk, cream, and butter.

Experiments were next made to determine the effect of the interval between the ingestion of the French weed and occurrence in the milk. Intervals from 2 to 12 hours were used between feeding and milking. It was found that the maximum effect appeared about 3 hours after feeding; by 5 hours, the obnoxious condition had largely disappeared; by 12 hours, no effect whatever was noticeable. After determining that the 3-hour interval apparently gave the most pronounced results, later experiments were all made on this basis. Varying amounts were fed to the same and to different cows including some producing milk liberally and some with a small production. Some indications were obtained that a more pronounced result was obtained with cows producing a small amount of milk. With cows producing at the rate of 25 pounds daily, 150 grams of the seed fed 3 hours before milking gave a pronounced effect.

Our experiments indicate that the minimum amount that will ordinarily result in a taste and odor that will be observed is between 90 and 150 grams of the seed when fed at an interval favorable for the appearance of the objectionable condition.

Effects of feeding green French weed plant

Complaints received by the authors concerning butter defects apparently due to French weed have been confined to the winter season when dry feed is used. The source of the trouble must be the ingestion of the seed with the grain or the dried plant with the hay. Some evidence has been obtained that either may occur. Statements in the literature indicate that trouble has been most frequently experienced during the grazing period.

To test the relation of the green plant to the appearance of the obnoxious flavor and odor in butter, a plot was sown to the French weed and experiments made by feeding the green plants in varying quantities. Difficulty was experienced in getting the cows to consume the green plants even when the cows were receiving no other green forage and had, for this reason, a keen appetite for ordinary green feed. No results were obtained from 300 grams of the green plant fed 3 hours before milking. When 610 grams were consumed, the same interval before milking, the typical French weed taste was readily detected in the milk, and, as usual, more pronounced in the cream and the most pronounced in the butter. The same results were found with larger amounts. There seems to be no question but that the objectionable taste will appear if the cow consumes a sufficient amount of the green plant; however, the extreme dislike for the green plant shown by every one of the 8 cows used suggests that the only danger from the green plant is when a shortage of other green forage in the pasture compels the cow to devour the objectionable French weed. It is possible that certain animals may develop a taste for the plant and in time come to eat enough to affect the milk regardless of the presence of other feed.

The detection of French weed seed in mixed feed

To samples of the mixed feed in use at the University dairy barn, ground French weed seed was added in amounts ranging from 0.5 to 3.0 per cent. Water at a temperature of approximately 50°C. was added sufficient to moisten the entire mass. In every case, the typical French weed odor was readily noticeable within a few minutes. The strength of the odor varied

with the amount of the ground seed added, but even with the 0.5 per cent sample, the odor could be readily noted and the irritating effects felt by the eyes. The odor from feeds containing 3 per cent of French weed was so pronounced that it was detected in the air of a large room by persons entering. The odor becomes most noticeable when the warm moist feed is enclosed for a short period in a partially filled container.

Commercial feeds composed partly of screenings often contain feeding molasses, largely to add to the palatability by covering up the bitter taste common to screenings. The odor of the molasses also obscures that of the French weed in making the test described. To remove this difficulty, the plan was adopted of washing the molasses from the feed and then noting if the typical odor of French weed could be observed. The procedure followed was to place about a pound of the feed to be tested in a cloth or towel of rather close texture. A gallon or more of warm water was poured, a portion at a time, on the feed and within a few minutes the free water was pressed out with the hands. The treatment was continued until the molasses appeared to be mostly removed. The washed feed was then put in a closed container and kept warm until examined within half an hour. By this means, the presence of French weed seed can be readily detected when even less than 0.5 per cent is present.

The simple plan of testing suggested will indicate the presence of French weed in quantities far below that apparently necessary to produce any serious taint in the milk. Our experiments indicate that a minimum of possibly 125 grams per day is necessary to produce noticeable flavor in the milk. If the screenings fed contained 2 per cent of French weed seed, 13 pounds would be required to carry 125 grams, which is suggested as the minimum likely to produce serious results in the quality of the milk.

Elimination of French weed taste

The prevention of food flavors in dairy products and the elimination of those that do gain access has attracted the attention of investigators. The work of Babcock (8) is of especial significance in this field. Apparently those flavors which are the result of

the presence of volatile oils are the most difficult to remove, presumably because they unite with the fat itself. A number of the common methods of reducing or eliminating feed flavors were tried with cream from the milk of cows, each receiving 225 grams daily of French weed in her grain ration. In each case, the treatment was applied to the cream before churning. The results reported are from an examination of the butter.

It was found that ordinary pasteurization of the cream somewhat reduced but did not eliminate the objectionable flavor. Affected cream was diluted with water and reseparated. The resulting butter was considerable better than the check, but the obnoxious flavor persisted. Neutralization of the cream after souring according to the procedure followed as a regular routine in many butter factories was without material effect. Heating the cream under vacuum, as practiced in a commercial way in some localities to remove objectionable odors and tastes, gave the best results but did not completely remove the French weed taste. The plan of using mineral oil, as recommended by McDonald and Crawford (9) was tried with the result that the French weed flavor was reduced but not eliminated.

A test was made to determine how small a percentage of French weed tainted cream might seriously affect butter quality. Tainted cream was obtained by feeding 227 grams of French weed daily to each of a group of cows. Cream from this milk was added to 3 lots of untainted cream at the rate of 6.5, 15, and 25 per cent. The 6.5 per cent of tained cream did not affect the butter so far as could be determined on examination by a Federal buttergrader, and it was scored 92. The lot containing 15 per cent of the tainted cream showed evidences of French weed flavor and was scored 91. The sample containing 25 per cent of the French weed cream showed more pronounced evidence of the objectionable taste and was scored 90.

Characteristics of the French weed taint

One of the interesting facts about French weed is the almost complete lack of any odor to the whole dry seed and the immediate development of an exceedingly disagreeable nauseating odor when

the ground seed is moistened. The unground seed develops the same odor when wet but not so quickly. Seeds of other plants of the *Cruciferae* show the same characteristic regarding the development of their typical volatile products in the presence of water.

One of the early investigators of this subject was Werenskiold (1). He states, as do later authorities, especially Gildermeister and Hoffman (10) that the seed of *Thalspi arvense*, as well as of other members of the mustard family, contain a glucoside known as sinigrin (myronate of potassium) and an enzyme known as myrosin. In the presence of water, the myrosin hydrolizes the glucoside known as sinigrin into mustard oil and other compounds. It appears that in the case of *Thalaspi arvense*, mustard oil and another compound, probably oil of garlic, are produced by the action of this enzyme. The mustard oil may be responsible for the irritating effect of the odor from French weed, but the oil of garlic gives the typical flavor.

An experiment by the authors indicated the correctness of this view. French weed seed and mustard seed were macerated and soaked over night. They were then distilled until 200 cc. of distillate was available for each. A series of cream samples were prepared and treated as indicated: (a) check; (*b*) 5 per cent of French weed distillate; (*b*), 5 per cent of mustard seed distillate; (*d*) 2 drops of mustard oil; (*e*) five drops of ether extract from ordinary garlic (*Allium sativum*). An examination of the butter churned from the five lots showed the odor as obtained from the French weed extract was not the same as that from mustard oil. Both showed the same irritating odor, but with the French weed this was combined with the same odor as that obtained from the common garlic.

DISCUSSION

The experiments reported show that either French weed seed or the growting plant may be responsible for an obnoxious taste and odor in the milk of a cow consuming these products. It might be expected that the trouble would be widespread in view of the extensive infestation of this weed in some districts in

Minnesota, the Dakotas, and the Prairie Provinces of Canada. The extreme distaste of livestock for the seed and forage is undoubtedly one explanation for the fact that more trouble does not occur. Apparently it is consumed only when the animal can not avoid doing so on account of the seed being in the grain mixture fed, or when the animal has a craving for green feed, and forage other than French weed is not available. This occurs usually in early spring and late fall. The common presence of this weed seed in grain screenings calls for care in feeding such products. The tests described offer a simple means of determining whether the objectionable seeds are present. Furthermore, the effect is observed only when what would appear to be a considerable amount, namely about 125 grams of the seed, is consumed. The effect of a smaller amount may be noted on the breath of the cow.

The experiments show that the French weed taint disappears completely within 12 hours after feeding. This indicates that the same practice as found effective in preventing the appearance of other food flavors will be effective, namely, feeding the suspected feed immediately after milking, with a reasonable certainty that the effects will be gone by the time for the next milking.

SUMMARY

Thlaspi arvense, known as penny-cress, French weed, or stinkweed, is a troublesome weed of the mustard family. It is the cause of a very objectionable taint in milk, cream, and butter.

This weed is a serious pest in the grain growing areas of Manitoba, Sasketchewan, Minnesota, the Dakotas, and Montana, and reports indicate that it is spreading.

The characteristic odor develops when the seed is in contact with water as the result of the action of an enzyme known as mycrosin, which hydrolyzes particular glucosides found in the plant. Apparently both oil of mustard and oil of garlic are formed.

It was shown experimentally that the ingestion by a lactating cow of French weed seed to an amount between 90 and 150 grams or of the green forage to the amount of 500 grams or more re-

sults in the characteristic taint in the milk. The odor is more marked in the cream than in the milk and is most pronounced in the butter. This may be taken to indicate that the volatile products unite chiefly with fat. The most pronounced results were obtained when the interval between feeding the seed and milking was 3 hours. The effect was reduced after an interval of 5 hours and completely gone by 12 hours. Feed containing French weed seed can be fed immediately after one milking without danger to the quality of the product from the next milking.

The common methods used to eliminate feed tastes from milk are only partially successful in removing the tainting of French weed. The taint could be detected in the butter from mixed cream when 15 per cent of the cream was tainted. French weed may be readily detected in mixed feeds in proportions as low as 0.5 per cent.

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GRADES AND METHODS OF ANALYSIS OF DRY SKIM MILK*

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The increased use of dry skim milk in the food industries has created a need for its proper classification by grades, so that the grade name may be taken as an index of quality. Formerly the quality of this product has been appraised by direct observation of its physical properties, by the results of selected laboratory determinations, or by a combination of both. Grading practices of the past have not been uniform due to the following: variation in the criteria accepted as a basis for grading; lack of adequate terminology to be applied to characteristics of quality; variations in laboratory practice; and undefined expression of the limits of probable errors in analysis.

The work of the Standards Committee of the American Dry Milk Institute consisted of collaborative work on laboratory technic, selection of the most suitable methods of analysis, the examination of a large number of samples and the correlation of the results of inspection and laboratory determinations with the more universally accepted trade demands of quality.

Three grades of quality have been established and these in turn are applied to each of three divisions named according to the method of their manufacture—spray, vacuum drum, and roller. A single standard for evaluating the quality of all dry skim milk would be simpler but owing to a variation in the relation of solubility to the several industrial applications of this product, the above division is deemed necessary at this time. Each of these divisions is subdivided into extra, standard, and third grade. It is not intended that classification under the

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terms of spray, vacuum drum, and roller should be in any way prejudicial to a particular process.

NORMAL VARIATION IN COMPOSITION OF DRY SKIM MILK

	per cent
Butterfat	0.7 – 2
Moisture	2 – 5
Acidity (lactic, reconstituted basis)	0.09– 0.20
Total protein (N × 6.38)	30 –38
Lactose (by polariscope)	47 –53
Total ash	7 – 8

The variations in total protein, lactose and total ash indicated above appear to result from seasonal changes in the raw milk and are not generally considered as important factors affecting quality. Inasmuch as heavy metals, chemical compounds or substances deleterious to health do not usually contaminate the product during the process of manufacture, no consideration was given to their detection.

Requirements for Extra Grade

1. General requirements. All dry skim milk for human consumption shall conform in all respects to the Federal Food and Drugs Act of June 30, 1906, and all subsequent rulings and decisions relative to the same as issued, from time to time, by the United States Department of Agriculture.

The factory and factory equipment used in the manufacture of this product shall be maintained in a clean, sanitary condition. No person affected with any recognized infectious, contagious or communicable disease, or who resides, boards, or lodges in a household in which there is a person affected with such disease, shall be employed or permitted to work in or about any part of the factory in which dry skim milk is manufactured.

Dry skim milk shall be made from freshly skimmed milk to which no alkali or other chemical has been added and which has been pasteurized in the liquid state either before or during the process of drying, at a temperature of 145°F. for 30 minutes or its equivalent in bacterial destruction.

The powder shall be reasonably uniform in composition and the color shall be white or light cream and free from a brown or yellow color, typical of overheated stock, and free from any other unnatural color.

Upon reconstitution it shall have a good flavor and be substantially free from sediment and specks.

2. Detailed requirements.—

	EXTRA GRADE SPRAY DRIED—MAXIMUM	EXTRA GRADE VACUUM DRUM DRIED—MAXIMUM	EXTRA GRADE ROLLER DRIED—MAXIMUM
Fat	1.5 per cent	1.5 per cent	1.5 per cent
Moisture	4.0 per cent	4.0 per cent	4.0 per cent
Titratable acidity (reconstituted basis)	0.16 per cent	0.16 per cent	0.16 per cent
Solubility index	1 cc.	2 cc.	12 cc.
Bacterial count (reconstituted basis)	30,000 per cc.	30,000 per cc.	30,000 per cc.
Sediment	No. 3	No. 3	No. 3

Flavor: In powdered form and on re-solution the products shall be sweet and clean and free from rancid, tallowy, fishy, cheesy, soapy or other decidedly objectionable flavors or odors.

Physical: It shall be free from all hard lumps and show no more black or foreign specks than in sediment no. 3.

Packing: The package shall be of such a character as to prevent contamination by dust, dirt or other foreign matter and to reduce to a minimum the possibility of absorption of foreign odors and moisture.

Requirements for Standard Grade

Any dry skim milk failing in one or more particulars to meet the requirements for Extra Grade shall be classed as Standard Grade, provided it meets all of the requirements for Standard Grade outlined below.

1. General requirements. Same as for Extra Grade.

2. Detailed requirements as follows:

	STANDARD GRADE SPRAY DRIED—MAXIMUM	STANDARD GRADE VACUUM DRUM DRIED—MAXIMUM	STANDARD GRADE ROLLER DRIED—MAXIMUM
Fat	2.0 per cent	2.0 per cent	2.0 per cent
Moisture	5.0 per cent	5.0 per cent	5.0 per cent
Titratable acidity (reconstituted basis)	0.2 per cent	0.2 per cent	0.2 per cent
Solubility index	1.5 cc.	5 cc.	15 cc.
Bacterial count (reconstituted basis)	100,000 per cc.	100,000 per cc.	100,000 per cc.
Sediment	No. 4	No. 4	No. 4

Flavor: In powdered form and on re-solution it shall comply with the requirements for Extra Grade with the exception that a storage flavor is permissible.

Physical condition: It shall be reasonably free from hard lumps and any large number of black specks or foreign matter.

Requirements for Third Grade

Any dry skim milk failing to meet the several requirements for Standard Grade especially with reference to moisture, acidity, sediment, or bacterial count, or those in which the odor or flavor indicate partial decomposition shall be classed as Third Grade and considered unfit for human consumption.

Revision of Grades

The above limits for grades are exceptionally broad and it is anticipated that changes will be made from time to time when available data indicate such changes will improve the quality of the product.

DISCUSSION OF DETERMINATIONS RECOMMENDED AS A BASIS FOR THE APPRAISAL OF QUALITY

Butterfat. Dry skim milk is manufactured to contain a minimum of butterfat. Excessive amounts have been observed to cause the development of an undesirable tallowy flavor and odor during storage. The point of maximum tolerance indicated in

the grading schedule was obtained by noting the effect of varying butterfat content on the resultant flavor.

Moisture. It has long been observed that skim milk powder which is high in moisture at the time of manufacture gradually becomes more insoluble upon storage. Such powders develop stale, tallowy, rancid and other off flavors. They develop lumpiness and more readily decompose during storage and frequently become questionably fit for human consumption. As dry skim milk is very hygroscopic, it is important to store it in a dry atmosphere to maintain low moisture content.

Various methods for determining moisture have been used and a comparison of results seem to indicate that more concordant results can be obtained by using the Toluol distillation method indicated herein.

Acidity. The determination of total acidity has been used freely in judging the quality of milk and milk products. It has been assumed to be a reliable criterion of the degree of bacterial development and although the titration values are not an actual measure of free acidity, the increment above the recognized normal value for the milk or milk products usually represents free lactic acid. Although high acidity of dry skim milk is usually accompanied by other unfavorable characteristics, it is thought desirable to suggest a uniform procedure for the estimation of titratable acidity and to include a maximum in the grading scheme. When the acidity of the raw skim milk is close to the souring point the resulting dried product is insoluble and tends to undergo other degenerative changes during storage. The acidity of the powder is expressed in terms of reconstituted skim milk because of the ready analogy to figures obtained on the raw product. As the degree of dilution and the amount of indicator readily affect the determination, strict adherence to the method is seemingly essential if comparable results are desired.

Solubility index. This procedure involves, in a measure, speed of solution as well as solubility. Solubility is a characteristic which has been beset with uncertain interpretation and by some it is thought that a skim milk powder of high solubility more nearly approaches on re-solution the physical characteristics of

the original skim milk. On the other hand there are others who believe that an insoluble powder will, when given sufficient time, finally reach as close to the physical condition of raw skim milk as does the more soluble powder in the shorter period. Some of the consumers of dry skim milk require powders that are readily soluble in water while others do not recognize this as being an important factor in the satisfactory use of the product. Solubility is a recognized characteristic of dry skim milk powders and can only be considered as an indication of quality in terms of each type of powder.

The most accurate and technically correct method for establishing solubility is that proposed by Lampitt and Hughes. This method, however, lacks simplicity although it affords an ultimate standard which will serve as a basis for more accurate investigations. It is believed that a quicker method and one possessing reasonable accuracy would be more useful.

Bacterial count. It is not definitely known if moderate variations in bacterial count affect the quality of dry skim milk. In the interest of sanitary practice and public health, it is believed that the consideration of bacterial count should be included for grading purposes. It is admitted that excessively high counts indicate that the original raw product was of poor quality, or a delay in the manufacturing process favored undue growth, or that contamination took place during manufacture or packaging. All of these are undesirable conditions and it is believed that a product of relatively low count will be more resistant to decomposition and aging effect than will the product having a relatively high count. It is probable that such decomposition starts before the milk is dried and action is continued in the dry state as a result of enzymatic or catalytic action.

Sediment. The undesirability of visible discoloration of the product by burnt particles or dust is self-apparent. Some of the more critical uses of dry skim milk reveal the presence of sediments which cannot be detected in the dried product by mere physical inspection. Foreign particles may enter the process with the raw milk or be picked up during the manufacture while burnt particles usually result from too high temperatures being

used during the drying process or by the local adherence of milk deposits to certain areas in the drying equipment. The sediment testing procedure proposed is similar to that rather widely used for whole milk. In testing dry skim milk of low solubility, sediment particles are apt to be obscured by the insoluble protein. In view of this, an alternative procedure for such milks has been suggested employing the principle of quiet sedimentation.

Color. Variations in the color of dry skim milk powder are not wide. If the milk is scorched or otherwise discolored, the condition is noticeable in the powder and may appear in the reconstituted milk.

Flavor and odor. The appraisal of flavor and odor as might be expected is subject to elements of variation and inaccuracy. Competence comes to the individual with experience and the cultivation of practiced discrimination. It is felt that by providing descriptive terms for the several objectionable flavors found in imperfect dry skim milk the appraisal of flavor and odor may be carried out in a more exact manner and imperfections related to their probable cause.

METHODS OF ANALYSIS

1. Method of sampling

On account of the readiness with which dry skim milk absorbs moisture, it is very important that all utensils and containers used for sampling be *clean* and *dry* and that the work be done as speedily as possible. Samples should always be taken at points beneath the exposed surfaces. For complete laboratory analysis 1 pound samples are required.

When it is necessary to prepare a composite sample, in order that the final sample will be representative of a given quantity of powder, several fairly good sized portions should be taken at different points and immediately transferred to a dry container in which they can be thoroughly mixed.

In barrel sampling, it is suggested that a long tube made of brass or nickel (iron should not be used) one and one half inches in diameter and sharpened at one end be thrust the full length of

the barrel at a point on the surface one third the distance between the centre and the edge.

The container should be large enough to hold all of the material sampled and readily permit of mixing the sample as described under "Preparing the Sample for Analysis." After mixing, immediately transfer the final sample to the shipping container and fasten the cover tightly. Only single or double friction top cans are recommended for shipping samples. Variations which may occur in a given quantity of powder can be more readily detected by examining separate individual samples rather than a composite sample and, in some cases, this practice is preferable.

Important—

Do not sample unless hands and clothing are clean and dry.

Do not sample in a humid atmosphere or in a room into which steam is being discharged.

Do not sample in a damp cold storage room or with utensils or containers which have just been brought from a colder room into a warm room.

Do not sample when your attention is being distracted by other duties.

2. Preparing the sample for analysis

If the sample of powder completely fills the container in which it is shipped, transfer all of it to another clean, dry container of about twice the size. With a suitable sized spoon turn over the contents by lifting it from the bottom of the can, at the same time slowly rotating the can at an angle of 45°. Mix the powder in this manner, by turning over a spoonful every second during a period of one minute. It is important, during mixing, to take the powder out of the lowest edge of the can. Precautions should be taken to reduce, to a minimum, the absorption of moisture during mixing; and as soon as the mixing is completed the sample should be returned at once to its original container and covered tightly. The above procedure can also be used for preparing composite samples by the use of a larger spoon and a larger container.

3. Moisture—Toluol distillation method

Apparatus required. 1 moisture tester complete, Bidwell and Sterling type (see Eimer & Amend Catalogue No. 28600) which

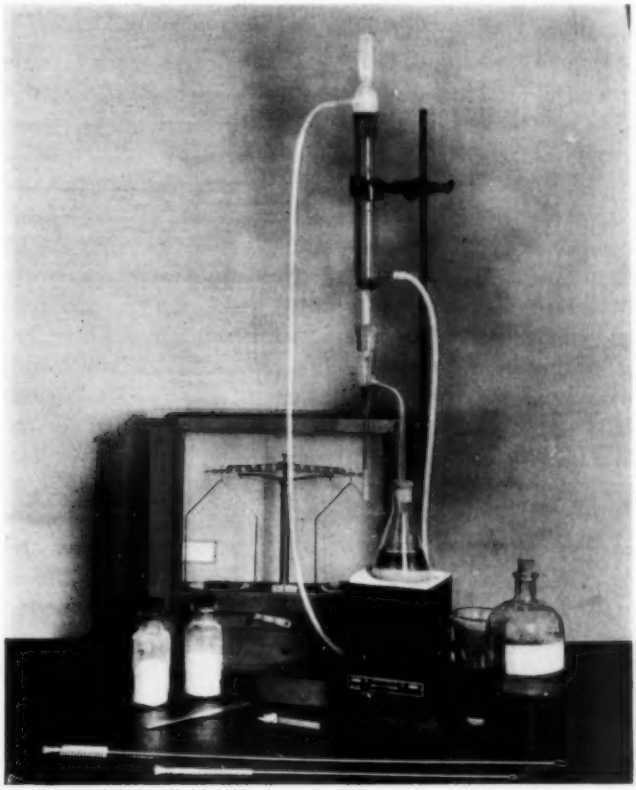

FIG. 1. EQUIPMENT FOR MOISTURE DETERMINATION

consists of a 250 cc. Erlenmeyer flask, 2—1 hole cork stoppers, distillation tube and condenser.

One electric hot plate or preferably electric heater (see Eimer & Amend Catalogue No. 24920).

Rubber tubing—suitable quantity for connecting water to the condenser (see Eimer & Amend Catalogue No. 30682—$\frac{1}{4}$ inside diameter). Ring stands and clamps for holding the apparatus.

Procedure. Introduce into the 250 cc. clean, dry Erlenmeyer flask 50 grams of spray process milk powder or 25 grams of roller process milk powder; immediately add sufficient toluol to cover the sample completely. Usually about 75 to 100 cc. is sufficient. Connect the flask with a condenser by means of the distillation tube. (For further information, see Ind. Eng. Chem., published February 25, 1925, page 147.)

Fill the receiving tube with toluol by carefully pouring through the top of the condenser. Bring to a boil rapidly by heating on the electric hot plate and then reduce the heat so that the Toluol will condense at the rate of about four drops per second. Thirty minutes after distillation has begun, dislodge any water particles in the condenser tube by means of a brush and wash down with 10 cc. of toluol. Twenty minutes later repeat and continue the distillation for an additional ten minutes. The flask should be shaken frequently after it is first put on the heater to prevent the powder from being burned and until the boiling is established and heat reduced to permit required rate of distillation.

Allow the receiving tube to come to room temperature. If any drops of water adhere to the side of this tube they can be forced down by means of a copper wire. Read the volume of water, estimating to hundredths of a cubic centimeter and calculate the percentage.

The condenser and receiving tube must be chemically clean in order to prevent any undue quantity of water adhering to the condenser and receiving tube. Wash with a solution of washing soda or soap powder and rinse with clear water. If necessary, fill with cleaning solution (sulphuric acid and potassium dichromate) and allow to stand over night. Rinse successively with water, alcohol, and ether and allow to dry in the oven. The tubes must receive this treatment after each determination, but the condenser usually once a week. If necessary, blank determinations should be made. It is important to use only moisture free Toluol and to make sure that the receiving tubes are accurately calibrated.

Note: The above method is to be regarded as the recommended one, but other methods which give comparable results may be employed. It has been determined that a vacuum oven operated at 100°C. in such a way that a current of air, dried by bubbling through sulphuric acid and admitted diagonally opposite the evacuation outlet at such a rate that the air is renewed every thirty seconds when the vacuum of the oven is maintained at a pressure of approximately 70 mm. of mercury, will give good checks with five hours of drying. Aluminum dishes 2 to 2½ inches in diameter, shallow form, with tightly fitting covers and one or two gram samples should be used. Equally good results have also been obtained with the vacuum oven at 100°C. operated under a pressure of 1 mm. of mercury for a period of four hours.

4. Butterfat

Apparatus required. Same as for Modified Roese Gottlieb or Mojonnier method.

Procedure. Weigh out 1 gram of the well mixed milk powder directly into a dry Mojonnier extraction flask, or in a suitable container whereby it can be easily transferred to a dry Mojonnier extraction flask or a dry Rohrig tube. If the latter is used, it should be of the Biesterfeldt type and should have a capacity of 20 cc. from the bottom up to the spigot. Add 8.5 cc. of warm water. Cork and shake vigorously until dissolved, warming slightly if necessary to room temperature. Add 1.5 cc. of strong ammonium hydroxide, shake again thoroughly. Add 10 cc. of ethyl alcohol 95 per cent, insert cork and shake again thoroughly. Add 25 cc. of ethyl ether, insert the cork and again shake thoroughly. Add 25 cc. of petroleum ether and shake as before. Allow the ether layer to separate by leaving the flasks or tubes at rest for twenty minutes or by centrifuging, turning the handle of the machine 60 times at a slow rate of speed. Pour or draw off the ether layer into a previously weighed aluminum dish. Evaporate on the hot plate or on a hot water bath at a temperature sufficient to allow complete evaporation, but not so high that spattering or too vigorous boiling will result. Add 4 to

5 cc. of alcohol to the residue left in the extraction flask or tube and mix thoroughly without inserting stopper. Add 15 cc. of ethyl ether, shake thoroughly, then 15 cc. of petroleum ether and again shake thoroughly. Separate the ether layer as before and pour off or draw off into the aluminum dish. Make a third extraction in exactly the same manner as the second, omitting any addition of alcohol. If necessary, carefully pour a few cubic centimeters of distilled water down the side of the tube just prior to pouring off third extraction to raise level of the aqueous layer, so ethers may be completely poured off. It is important that at no time should any of the aqueous layer be allowed to run into the aluminum dish.

The time required for shaking, after adding the first portion of alcohol and subsequent additions of either ethyl or petroleum ether should be not less than one-half minute, providing the shaking is very vigorous.

Each time after pouring off the ether layer, the lip of the extraction flask or spigot should be rinsed with petroleum ether, allowing the rinsings to run into the aluminum dish. Petroleum ether should have a boiling point below 60 degrees and both petroleum and ethyl ether should be free from residue on evaporation.

After the ether is entirely evaporated from the aluminum dish, it should be placed in the Mojonnier oven for five minutes with the temperature at exactly 135°C. or in boiling water oven for thirty minutes or longer, as required, to bring it to constant weight. In weighing the dishes both when empty and when containing the extracted fat, they should be at a temperature preferably within 1° of the temperature of the balance; and should never have more than 2° difference. Fresh sulphuric acid appears preferable as drying medium in the desiccator in which the dishes are cooled. If desired, confirm the purity of the fat by dissolving in a little petroleum ether. If a residue remains, remove fat completely by solution and washing out dish with petroleum ether. Dry the residue and deduct weight. Blank all reagents.

5. Solubility index

Apparatus required. A supply of 50 cc. conical sediment tubes specially graduated in one-tenth divisions from 0 to 1 cc., in 0.2 divisions from 1 to 3 cc., and 0.5 divisions from 3 to 15 cc., and also at the 50 cc. mark.

Suitable glass syphon tubes.

Hamilton Beach electric mixer.

FIG. 2. EQUIPMENT FOR SOLUBILITY INDEX

Two hundred or 300 cc. glass beakers or glass tumblers of equal capacity.

Fifty cubic centimeter wide mouth pipette.

Wood adapter or rubber pad to hold the conical sediment tube in centrifuge cup.

Procedure. Add 20 grams of dry skim milk to 200 cc. of distilled or filtered tap water at a temperature of 60°F. and imme-

diately agitate vigorously by the use of a Hamilton Beach electric mixer for thirty seconds. If necessary transfer immediately to a suitable sized container so that after the foam has separated it can be readily removed from the surface by means of a spoon. Allow to stand at room temperature for a period of five minutes and then remove all the foam from the surface with a tablespoon. Agitate the milk thoroughly with the spoon by stirring rapidly for fifteen seconds and immediately pour 50 cc. into the specially calibrated conical tube. Place the conical tube at once in a hand or motor driven centrifuge and whirl for fifteen minutes at the normal R.P.M. Syphon off the supernatant liquid to 7 cc. mark if possible. If the amount of sediment is so large that this cannot be done, then syphon it within 2 cc. of the surface of the sediment, being careful not to draw any of the sediment into the syphon tube. Add about 25 cc. of distilled or filtered tap water, temperature 60°F., and shake thoroughly so as to dislodge all of the sediment from the lower portion of the tube. If necessary use a metal wire to dislodge the sediment. Fill to the 50 cc. mark with distilled or filtered tap water, temperature 60°F., invert and shake the tube so as to mix the contents thoroughly and whirl a second time in the centrifuge for a period of fifteen minutes. Hold the tube in an absolutely vertical position so that the upper level of the sediment is in line with the eye. Read the amount of sediment in the tube and record the results in cubic centimeters. The upper surface of the sediment is easily seen when the tube is held toward a strong source of light.

Note: It is important that the water used in determining solubility should be free from sediment and always at a temperature of 60°F. Periods of stirring and centrifuging must also be observed exactly if reasonable checking results are desired. Do not use a steam turbine centrifuge and frequently check the speed to make sure that normal R.P.M. is maintained. The normal R.P.M. of the Babcock centrifuge varies according to the diameter of the wheel according to the following table:

Diameter of wheel	*Normal R.P.M.*
10 inches	1074
12 inches	980
14 inches	909
16 inches	848
18 inches	800
20 inches	759
22 inches	724
24 inches	693

6. *Titratable acidity (reconstituted basis)*

To 200 cc. of neutral or distilled water, add 20 grams of dry skim milk. After the powder is apparently in solution, withdraw by means of a pipette 17.6 cc. and transfer to a white porcelain cup, or similar container. Rinse out the same pipette with 17.6 cc. of neutral or distilled water and add it to the skim milk in the cup. Then add 0.5 cc. of a 0.5 per cent solution of phenolphthalein and titrate with N/10 sodium hydrate until a faint pink color persists for thirty seconds. The number of cc. of sodium hydrate used divided by 20 indicates the percentage of acidity of the reconstituted skim milk in terms of lactic acid. The phenolphthalein solution should be prepared by dissolving one-half gram of dry phenolphthalein powder in 50 cc. of ethyl alcohol and adding 50 cc. of water after the powder has gone into solution.

7. *Foreign sediment and black specks*[1]

Apparatus required. Wisconsin sediment tester using 1¼ inch disc.

Supply of 1¼ inch cotton discs.

Twelve ounce Bell top tumblers (2 inches diameter at base).

Procedure. One pint of restored milk containing 10 grams of the powder in 100 cc. should be passed through a 1¼ inch disc sediment tester and the disc examined, for foreign sediment, burnt particles, and compared with standard disc illustrations. If re-dissolved powders will not pass through the sediment tester, it

[1] On account of difficulty in printing disc charts, photographic copies will be supplied on request to the American Dry Milk Institute, Inc., 221 North La Salle Street, Chicago, accompanied by 80 cents to cover cost.

is suggested that a one-half pint sample be allowed to stand in a 12 ounce bell-top tumbler (diameter of base 2 inches approximately) for five hours and amount of sediment in the bottom of the tumbler be compared with the standard tumbler illustration.

8. Flavor and odor

The dry skim milk should be examined for flavor and odor one hour or more after restoring to normal liquid form. It should be classified as: good, fair, or bad, off odors and flavors being characterized by such terms as—storage, stale, tallowy, rancid, cheesy, putrid, cowy, fishy, metallic, cooked or flat.

9. Bacterial count (reconstituted basis)

A. Completely dissolve 10 grams of a well mixed powder in 100 cc. of sterile, distilled water. It is suggested that this be done by weighing 10 grams directly into a 6 ounce sterile bottle containing 100 cc. of sterile water and furnished with a sterile rubber stopper. It is important that the mixture be thoroughly shaken so as to make sure that the powder is completely dissolved.

B. After reconstitution as in *A*, follow the procedure as outlined for the microscopic colony count (plate method) as published in the Standard Methods of Milk Analysis of the American Public Health Association, Fifth Edition, 1927. It is important to note that the final count is to be expressed as the plate count per cubic centimeter of the reconstituted solution.

ACCURACY OF RESULTS

It is not infrequent that in noting the results of laboratory analyses one observes that variations occur when duplicate determinations are made. He is prone to feel that the analyses cannot be relied upon unless results check exactly. The fact that the method of analysis has been recommended by a Committee who have given the details of the method careful consideration and study does not insure the fact that it is absolutely exact. It simply indicates that it is the best method they are able to recommend. In order that one may appreciate the limitations

of results that can be obtained upon duplicate analysis, a study has been made of the possible variations that may be expected and for information and guidance they are indicated as follows:

a. When determinations are made on the same sample in a given laboratory:

1. Moisture should check within 0.2 per cent.
2. Fat should check within 0.1 per cent.
3. Acidity of reconstituted skim milk should check within 0.01 per cent.
4. Solubility Index should check within:

0.1 cc. where the total sediment is not greater than 1 cc.

0.1 cc. to 1 cc. where the total sediment is between 1 cc. and 10 cc. The greater the sediment the greater the variation.

2 cc. where the total sediment is above 10 cc.

b. When determinations are made on duplicate samples in different laboratories:

1. Moisture should check within 0.4 per cent.
2. Fat should check within 0.3 per cent.
3. Acidity of reconstituted skim milk should check within 0.02 per cent.
4. Solubility Index should check as above.

The method of determining bacterial count has been adopted because it is the procedure generally followed for making bacterial counts in all dairy products. Wide variations in count are frequently noted and it is suggested that two or more determinations be made each on a different sample.

STUDIES ON THE CHEMICAL COMPOSITION OF BOVINE BLOOD*

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With the development of methods for the analysis of blood, a valuable tool has been placed in the hands of experimenters in physiology and nutrition and of physicians in the clinical laboratory. At the present time a great deal of information is available on the abnormal composition of human blood in disease. A knowledge of the composition of normal blood was obviously a preliminary necessity for the interpretations of these abnormalities.

In order to extend the use of blood analysis to cattle, normal values must first be established for the various blood constituents. A considerable amount of data along this line are available at the present time. Abderhalden (1) in 1898 published a rather complete analysis of the blood of various domestic animals including cattle. More recently important contributions have been made by Meigs, Blatherwick and Cary (22), Blatherwick (6), Robinson and Huffman (23) and Hayden and his co-workers (18, 19, 20). In the course of our study on the composition of the blood of dairy cattle under various conditions we have accumulated considerable data on normal animals which we have assembled in this paper. It is hoped that these data may add to the present knowledge of normal bovine blood and may be of value to future workers in the field of bovine physiology, nutrition, and pathology.

METHODS

Blood samples were obtained from the jugular vein with as little stasis as possible. Two samples were always taken, one in a

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small Erlenmeyer flask, with no oxalate, for the preparation of serum in which calcium and phosphorus were determined; the other in a large test tube under paraffin oil, using 40 mgm. of lithium oxalate to prevent clotting. About 75 cc. of blood were collected in this manner to be used for the determination of hemoglobin, carbon dioxide and the substances included in the Folin-Wu system of blood analysis. The portion of the sample used for the determination of carbon dioxide was centrifuged immediately under paraffin oil and the plasma removed.

Hemoglobin and carbon dioxide were determined by the gasometric methods of Van Slyke (25, 26). For the estimation of non-protein nitrogen, creatine, creatinine, uric acid, sugar and chlorides, a protein free filtrate was prepared according to the procedure of Folin and Wu (12). Non-protein nitrogen, creatine and creatinine were determined by the original procedure of Folin and Wu (12). Uric acid was determined by Benedict's method (5), sugar by the method of Folin and Wu (13) and chlorides by the method of Whitehorn (28). Urea nitrogen was determined on whole blood by the method of Van Slyke and Cullen (27). Phosphorus was determined in three forms, namely, inorganic, hydrolyzable organic and total acid soluble phosphorus, using the Briggs modification (7) of the Bell-Doisy method (2) with some slight modifications of procedure. Calcium was determined by Clark and Collip's modification (8) of the Kramer and Tisdall method (21, 24).

EXPERIMENTAL

The work embodied in this report includes the analysis of 59 samples of blood taken from the dairy herd of the Pennsylvania State College. The animals include calves and adults of various ages and breeds. The animals received the regular ration used for the dairy herd. The blood samples were taken during the late summer, fall, and winter and no attempt was made to study seasonal variations. The results, which are averages of duplicate determinations, are presented in tables 1 and 2. The animals are grouped into 4 groups on the basis of age. In the first group are those animals less than one month of age. In the second group

TABLE 1

Showing the composition of the blood of normal dairy cattle

ANIMAL NUMBER	SEX*	BREED*	AGE*	WHOLE BLOOD								SERUM				PLASMA
												Phosphorus				
				Hemoglobin	Non-protein nitrogen	Urea nitrogen	Uric acid	Creatine	Creatinine	Sugar	Chlorine as sodium chloride	Inorganic	Inorganic plus hydrolysable organic	Total acid soluble	Calcium	Plasma bicarbonate CO_2
Animals ranging from 1 to 28 days of age																
				per cent normal	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*volumes per cent*
1312	M.	A.	0- 0- 1		30.80	4.40	1.97	4.81	1.62	123.0	503				12.93	
1308	M.	H.	0- 0- 6		25.91		1.59	4.67	1.55	96.0	484					
1310	M.	H.	0- 0- 7		35.42	15.63	2.40	4.42	1.82	89.0	473				13.40	
1323	F.	A.	0- 0- 9	121.7	42.14		3.10	4.06	1.49	105.7	457	5.23	5.72	7.80	12.52	52.2
1327	F.	H.	0- 0-10	104.0	26.99	19.80	1.53	3.83	1.73	99.8	440	5.05	7.60	8.44	13.46	58.9
1321	F.	A.	0- 0-11	90.8	37.15	8.21	2.19	3.35	1.47	98.4	471	4.55	5.75	6.05	11.76	62.6
1309	M.	A.	0- 0-13		31.55	10.53	2.13	5.57	1.41	105.0	492				12.88	
1307	M.	H.	0- 0-16		30.77	10.00	1.76	4.07	1.63	138.0	505				12.60	
1324	F.	G.	0- 0-17	99.2	20.67	12.68	1.71	3.65	1.54	86.8	426	5.30	7.90	8.57	15.00	65.5
1323	F.	A.	0- 0-28	96.7	26.97	15.10	1.91	3.33	1.57	98.9	434	5.20	6.45	8.03	14.48	62.6
Animals ranging from 1 to 5 months of age																
1321	F.	A.	0- 1- 7	79.8	27.62	15.59	1.64	4.78	1.61	104.5	479	6.17	7.50	8.47	14.49	65.5
1308	M.	H.	0- 1-29		29.92	8.62	2.21	4.27	1.38	81.6	515	3.52			12.87	64.5
1000	M.	H.	0- 2-		26.64	14.05	2.86	2.49	1.53	64.3	536					61.7

1168	F.	H.	0- 2-	89.9	33.38	6.21	2.96	4.08	1.52	119.5	448	5.36	6.85	8.24	16.18	64.5
1169	F.	H.	0- 2-	105.4	34.47	12.99	2.00	4.95	1.60	142.8	460	4.77	6.57	7.59	13.81	56.0
1182	M.	H.	0- 2-	85.4	36.82	11.30	1.70	4.58	1.94	138.6	506				11.19	76.8
1168	F.	H.	0- 2-	98.5	31.86	7.12	1.94	6.99	1.45	90.5	487	5.72	7.70	7.91	12.64	65.3
1169	F.	H.	0- 2-	100.9	30.88	10.08	2.00	6.86	1.28	100.0	514	5.15	7.08	7.77	11.49	58.7
1170	F.	H.	0- 2-	95.1	31.58	7.57	1.85	5.83	1.60	120.7	461	3.95	6.28	7.07	11.59	73.0
1307	M.	H.	0- 2- 2		25.73	7.99	1.55	4.32	1.36	71.7	505	3.09			11.99	59.8
1296	M.	A.	0- 2- 2		30.52	12.79	2.00	4.25	1.27	77.0	501				14.30	
1292	F.	J.	0- 2-14	70.3	29.21		1.56	4.48	1.43	73.0	495				12.62	65.5
1302	M.	H.	0- 2-15		27.90	18.91	1.70	4.01	1.42	78.3	546	3.29	3.47		12.96	71.8
1303	M.	H.	0- 2-15		26.38	21.64	1.63	4.37	1.42	77.7	546	3.32	3.46		13.31	67.1
1301	F.	H.	0- 2-21		26.83	13.55	2.08	4.34	1.37	119.9	492	3.41			11.61	67.3
1293	F.	A.	0- 2-22	79.4	29.30	14.30	2.20	7.78	1.45	87.2	540				12.60	52.5
1291	M.	J.	0- 2-25	93.6	26.52	11.70	1.54	3.67	1.28	82.0	495					58.6
1292	F.	J.	0- 2-28		25.00		1.90	4.15	1.25	71.0	506				12.24	
1293	F.	A.	0- 2-28		23.60		1.92	3.67	1.41	80.0	510				10.00	
1290	F.	A.	0- 3- 6	73.3	22.20		1.80	3.89	1.32	72.2	499				11.62	55.1
1288	M.	A.	0- 3-14	86.9	28.00	11.20	1.95	3.61	1.35	75.0	490				10.55	56.7
1287	M.	A.	0- 3-18	86.9	30.18	12.41	1.85	3.83	1.36	67.8	487				12.20	59.8
1290	F.	A.	0- 3-23		25.75	11.00	2.05	2.90	1.19	93.0	513					
1291	M.	J.	0- 4- 5		25.00	10.43	1.96	3.90	1.33	87.3	470				13.80	
1287	M.	A.	0- 4- 8		24.96	11.10	2.05	4.54	1.31	69.0	488				14.16	
1283	F.	A.	0- 4- 8	77.1	27.96	11.99	1.65	3.99	1.32	71.8	496				11.00	63.6
1280	M.	J.	0- 4-15	81.4	26.91		2.20	4.25	1.45	75.0	491				9.96	64.3
1283	F.	A.	0- 4-22		27.48	13.40	1.68	4.47	1.25	66.0	495				11.70	
1280	M.	J.	0- 5- 5		27.53	10.00	1.45	4.04	1.21	82.2	488				11.76	
			Animals ranging from 6 to 10 months of age													
1292	F.	J.	0- 6-12	101.2	33.06	13.93	3.22	4.42	1.38	59.6	506	5.45	6.70	7.50	12.55	54.1
1271	F.	G.	0- 6-16	86.2	34.40	15.00	1.71	7.11	1.33	65.5	511				12.20	57.5
1280	M.	J.	0- 6-17		29.13	14.28	1.72	4.06	1.36	90.0	506	3.50			13.40	61.7

TABLE 1—*Concluded*

ANIMAL NUMBER	SEX*	BREED*	AGE*	WHOLE BLOOD							SERUM				PLASMA
											Phosphorus				
				Hemoglobin	Non-protein nitrogen	Urea nitrogen	Uric acid	Creatine	Creatinine	Sugar	Chlorine as sodium chloride	Inorganic	Inorganic plus hydrolyzable organic	Total acid soluble	Calcium	Plasma bicarbonate CO_2
Animals ranging from 6 to 10 months of age—Concluded															
				per cent normal	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*volumes per cent*
1271	F.	G.	0– 6–27		31.90	19.07	2.13	3.56	1.26	78.0	482				11.76	
1266	F.	J.	0– 8– 7		26.43	14.65	1.80	3.89	1.24	67.0	458				11.65	
1265	F.	H.	0– 8–10		32.43	16.42	1.62	3.82	1.26	76.5	481				12.80	
1325	F.	H.	0– 8–10	110.2	31.45	10.08	2.32	4.54	1.51	127.0					14.70	38.1
1266	F.	J.	0– 8–14		35.72	20.00	2.10	2.98	1.30	75.0	491				12.46	
1259	F.	G.	0– 8–19	102.1	42.10	18.00	1.92	4.77	1.39	67.0	480				11.83	61.6
1254	F.	J.	0– 8–29		26.78	13.90	1.52	3.72	1.21	69.6	471				12.60	
1259	F.	G.	0– 9– 0		35.20	17.00	2.10		1.38		478				12.87	
1254	F.	G.	0– 9– 6		35.98	17.93	1.91	3.41	1.32	70.0	476				12.06	
1250	F.	H.	0– 9–18		32.93	14.50	1.50	3.77	1.30	73.8	528				11.50	
1250	F.	H.	0–10– 2		25.60	13.97	1.90	4.08	1.45	74.0	510				11.60	
Animals ranging from 1 to 9 years of age																
1237	F.	G.	1– 0– 4		29.20		2.28	4.05	1.41	68.4	515				12.87	
1233	F.	J.	4– 2–27	81.1	34.20	13.30	2.40	3.83	1.37	51.0	509	3.75	5.10	5.75	13.40	56.0
1233	F.	J.	4– 3–25	109.0	32.20	13.52	2.49	5.01	1.66	47.0					14.78	38.1
1011	F.	B.S. + J.	4– 9–19	91.8	35.76	9.18	2.20	2.80	1.48	45.7	532	3.50	3.90	5.30	12.46	55.1
1011	F.	B.S. + J.	4–11–17	92.4	32.20		2.47	4.11	1.65	43.2					13.49	31.5
838	F.	J.	9– 3–10	103.6	29.23	10.26	2.92	3.79	1.31	51.8					12.88	36.2

* M. = male; F. = female; A. = Ayrshire; H. = Holstein; G. = Guernsey; B.S. = Brown Swiss; J. = Jersey.
The final numbers refer to age in years, months and days.

are animals from one to five months of age. The third group includes those from six to ten months of age, and the last group includes animals from one to nine years of age.

In table 2 is given a summary of results given in table 1, with averages for various age groups, for male and female, and for the different breeds. The results obtained by other investigators are also given in this table. The results of other investigators have been recalculated in some cases so that all the data presented in table 2 might be comparable.

Hemoglobin. Tables 1 and 2 show the results of 29 analyses of blood for hemoglobin. The results are expressed as per cent normal and the values range from 70.3 to 121.7, the average value being 92.9. The hemoglobin content appears to be highest in the animals less than one month of age. Females show a higher value than males and Holsteins show the highest average of the various breeds. Our results agree well with those of Hayden and Fish (18).

Non-protein nitrogen. Our figures for non-protein nitrogen are in very close agreement with those of other investigators. Fifty-nine determinations give an average of 30.07 mgm. per 100 cc. of blood with values ranging from 20.67 to 42.14 mgm. The averages for the various groups of animals are all very close to the total average.

Urea nitrogen. Fifty estimations in the course of our work have given an average of 12.94 mgm. of urea nitrogen per 100 cc. of blood, with minimum and maximum values of 4.40 and 21.64 mgm. respectively. The low value of 4.40 mgm. was obtained on a sample from a calf one day old. The averages for the various groups differ only slightly from the grand average except in the case of the Brown Swiss-Jersey cross. It should be noted that this value of 9.18 is for one individual. Our results agree with those of most of the other investigators.

Uric acid. For 59 determinations of uric acid, using the direct method of Benedict (5), we report an average of 2.08 mgm. which exactly duplicates the findings of Hayden and Sholl (19). Our values range from 1.50 to 3.22 mgm. The only group of animals which differs decidedly from this value is that of the older animals,

TABLE 2

Showing a summary of the data given in table 1 and also the results obtained by other workers

Description	WHOLE BLOOD								SERUM				PLASMA
									Phosphorus				
	Hemoglobin	Non-protein nitrogen	Urea nitrogen	Uric acid	Creatine	Creatinine	Sugar	Chlorine as sodium chloride	Inorganic	Inorganic plus hydrolysable organic	Total acid soluble	Calcium	Plasma bicarbonate CO_2
	per cent normal	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*Volumes per cent*
Average for all animals	92.9	30.07	12.94	2.08	4.30	1.42	84.1	492	4.46	6.12	7.46	12.63	58.9
Maximum for all animals	121.7	42.14	21.64	3.22	7.78	1.94	142.8	546	6.17	7.90	8.57	16.18	76.8
Minimum for all animals	73.3	20.67	4.40	1.50	2.49	1.19	43.2	426	3.09	3.46	5.30	9.96	31.5
Average for animals less than 1 month of age	102.5	30.83	12.04	2.03	4.16	1.58	105.0	469	5.06	6.68	7.78	13.22	60.4
Average for animals from 1 to 5 months of age	86.9	28.28	11.91	1.91	4.49	1.40	87.6	499	4.54	6.11	7.84	12.40	63.3
Average for animals from 6 to 10 months of age	99.9	32.36	15.62	1.96	4.15	1.33	76.4	491	4.48	6.70	7.50	12.42	54.6
Average for animals from 1 to 9 years of age	95.6	32.13	11.56	2.46	3.93	1.48	51.2	519	3.62	4.50	5.52	13.31	43.4
Average for males	86.8	28.83	12.07	1.91	4.19	1.45	89.4	501	3.34	3.47		12.62	63.9
Average for females	93.7	30.71	14.19	2.05	4.34	1.41	81.9	487	4.92	6.51	7.46	12.65	56.7
Average for Aryshires	89.1	29.14	11.69	2.01	4.30	1.40	87.2	491	5.47	6.26	7.59	12.48	59.0
Average for Holsteins	98.7	30.19	10.73	1.96	4.51	1.51	99.2	496	4.24	6.13	7.84	12.73	63.1
Average for Guernseys	95.8	32.78	16.61	1.96	4.63	1.38	72.6	481	5.30		8.57	12.66	61.5
Average for Jerseys	91.5	29.06	13.27	2.06	4.01	1.34	70.1	491	4.23	6.57	6.63	12.62	54.3
Average Brown Swiss-Jerseys	92.1	33.98	9.18	2.34	3.41	1.57	44.5	532	3.50	3.90	5.30	12.98	43.3
Hayden and Fish (18)	89.24	29.79	12.21	2.04 0.623*		1.20	46.52	444.1	5.7		9.29	10.88	

Folin and Dennis (10)		24.00	14.00	0.2*									
Greenwald (14)		32.00											
Hayden and Tubangui (20)		31.00	18.8	2.12		1.84	96.00						
Hayden and Sholl (19)		30.11	11.8	2.08		1.37	51.75	516.1					
Benedict (4)				0.5*									
Folin and Dennis (11)					10.44	2.00							
Greenwald and McGuire (15)					7.23	2.58							
					2.35†	1.61†							
Abderhalden (1)								507.6	8.47			11.94	
Robinson and Huffman (23)								542.8	5.87			11.0	59.2
Hart, et al. (16)									3.94			16.25	
Meigs, Blatherwick, and Cary (22)									5.38			10.38	
Hart, Steenbock, and Humphrey (17)												10.00	
Blatherwick (6)													61.5‡
													73.0§

* Indirect method.
† Their own method.
‡ Cows.
§ Calves.

from one to nine years of age, where an average of 2.46 mgm. was found.

Our results check well with those of investigators who have used the direct method for the determination of this constituent. Folin and Dennis (10), using a method in which the uric acid is isolated from a blood filtrate as the silver salt before being determined colorimetrically, report a much lower value of 0.2 mgm. Benedict (4) using his modifications (3) of this technique obtained a value of 0.5 mgm. He pointed out that the uric acid determined by this method is "free" uric acid and that this is only a small fraction of the uric acid present. The remainder exists in a "combined" form from which it is easily set free by hydrolysis with acid. Davis and Benedict (9) have shown that the uric acid is combined with a pentose. After hydrolysis of a protein-free blood filtrate, he obtained values as high as 6.7 mgm. It should therefore be pointed out that our results and those of the other investigators whose results are reported in table 2 very likely do not represent true uric acid values but only that which is set free under the conditions of the analysis.

Creatine. In our work, we have found an average of 4.30 mgm. of creatine per 100 cc., with values ranging from 2.49 to 7.78 mgm. The oldest animal group showed the greatest variation from the average, with an average value of 3.93 mgm. The low value of 3.46 for the Brown Swiss-Jersey cross is possibly not significant since there is only one individual in this group. It should be noted that we have reported actual creatine values and not creatine plus creatinine.

Our results do not agree with those of other investigators. Folin and Dennis (11) report 10.44 mgm. and Greenwald and McGuire (15), using Folin's technique, report 7.23 mgm. The latter investigators feel that Folin's method gives high results and they report a value of 2.35 mgm. using a new method.

Creatinine. From 59 determinations for creatinine we have obtained an average of 1.42 mgm. per 100 cc. with values ranging from 1.19 to 1.94 mgm. With the calves the values decrease with age. Holsteins give the highest average and Jerseys the lowest. There is considerable variation in values reported by other investigators.

Sugar. Our work has given an average of 84.1 mgm. of sugar for 58 determinations with values ranging from 43.2 to 142.0 mgm. A rather striking feature of the data is that blood sugar values decrease as the age of the animal increases. The youngest group gave an average of 105.0 mgm. while the group of older cows gave an average of 51.2 mgm. In other work we have always found low blood sugar values with cows which are lactating heavily. Our results for cows agree very closely with those found by Hayden and Scholl (19).

Chlorides. As a result of 55 analyses, we report an average of 492 mgm. of sodium chloride per 100 cc. The results range from 426 to 546 mgm. The animals in the group less than 1 month of age gave an average of 469 mgm. which is the group showing the greatest variation from the total average which we report. The value of 532 mgm. for Brown Swiss-Jersey is the result for one animal. Our average is about an average of results reported by other investigators.

Inorganic phosphorus. As a result of 20 analyses, we obtained an average of 4.46 mgm. of inorganic phosphorus per 100 cc. of serum, with values ranging from 3.09 to 6.17 mgm. The conspicuous thing about these results is that as the age increases the phosphorus values decrease. The group of youngest calves gives an average of 5.06 mgm., while the cows give a value of 3.62 mgm. per 100 cc. of serum. Females give a higher average than males and Ayrshires and Guernseys give higher averages than Holsteins and Jerseys. There is considerable variation in the values reported by other investigators.

Inorganic plus hydrolyzable organic phosphorus. In the work reported on blood phosphorus, we have found that in most cases only inorganic phosphorus has been considered. Meigs, Blatherwick and Cary (22) have reported on phosphorus distribution in blood plasma, but the distribution is not analogous to that which we report.

As a result of 16 determinations of inorganic plus hydrolyzable organic phosphorus in blood serum, we have found an average of 6.12 mgm. per 100 cc. with values ranging from 3.46 to 7.9 mgm. Here, again, the very young animals showed higher values than

the more mature ones. The average for females is nearly twice that for males. By deducting the average inorganic phosphorus from the average of the combined forms, we get 1.17 mgm. for the average hydrolyzable organic phosphorus in 100 cc. of blood serum.

Total acid soluble phosphorus. Hayden and Fish (18) report an average of 9.29 mgm. of acid soluble phosphorus per 100 cc. of serum as a result of 29 determinations on cows.

From 14 determinations we report an average of 7.46 mgm. of total acid soluble phosphorus per 100 cc. of serum, with values ranging from 5.30 to 8.57 mgm. Our results show a considerably higher average for calves than for older animals. Our results on cows are much lower than those reported by Hayden and Fish (18).

From all our results on the various forms of phosphorus in blood serum, it is clear that the serum of young growing animals has a higher phosphorus content than that of mature animals. This is to be expected when one considers the phosphorus requirement for bone-building in young animals. The lower phosphorus content of the serum of mature cows is also reasonable when one considers the large amounts of this element which are secreted into the milk.

Calcium. The results which we report for calcium gave an average of 12.63 mgm. per 100 cc. of serum for 55 analyses, with a range of 9.96 to 16.18 mgm. The results indicate no noticeable differences in the calcium content of young and mature animals. The average for 5 cows from four to nine years of age is 13.31 mgm., while that for 9 calves ranging from one to twenty-eight days of age is 13.22 mgm. Our results are quite definitely higher than those of all the other investigators except Hart and his co-workers (16).

Carbon dioxide. In our work we have determined carbon dioxide on 36 individuals with results ranging from 31.5 to 76.8 cc. per 100 cc. of plasma. The average value is 58.93 cc. A noticeable observation is that the plasma of cows' blood binds less carbon dioxide than that of calves. This has also been noted by Blatherwick (6). Males give a higher average than females. Our

average value is very close to that found by Robinson and Huffman (23).

SUMMARY

Fifty-nine samples of blood from dairy calves and cows have been analyzed for hemoglobin, non-protein nitrogen, urea nitrogen, uric acid, creatine, creatinine, sugar, chlorides, phosphorus, calcium and carbon dioxide binding capacity. The results are given in tables 1 and 2.

The authors wish to acknowledge the coöperation of Dr. S. I. Bechdel of the Department of Dairy Husbandry in allowing us free access to the dairy herd.

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BOOK REVIEWS

Milk and Milk Products. Clarence Henry Eckles, Willes Barnes Combs, and Harold Macy, University of Minnesota. Published by McGraw-Hill Book Company, New York, 1929.

This book was prepared to serve as a text for the first course in dairy manufactures in colleges of agriculture. It is based upon the experiences of the authors in teaching this course. Their accomplishments in teaching will recommend this book to everyone concerned with the teaching of the introductory course in dairying.

The book covers the composition and properties of milk, a brief survey of bacteriology, methods of testing, the processing of market milk, and the manufacture of butter, cheese, ice cream, dry milk, condensed milk, and milk by-products.

The introduction, giving the history and development of the dairy industry, is especially interesting even to an advanced student. The authors are to be commended for having omitted the customary chapter on the physiology of milk secretion and the over-used diagramatic section of a cow's udder. The organization of material is excellent except that the appendix contains tests even though it is preceded by a chapter on "Miscellaneous Tests." There is some repetition of tests.

It is doubtless essential to make statements in introductory books without all the necessary qualifications. One of the purposes of advanced courses is to explain why the definite, simple statements made in the elementary course are not absolutely true. In this book one might desire more detail concerning certain statements, as, for example the "slight practical significance" of the germicidal action of milk in view of its extensive use in not cooling morning's milk; the delivery of 18 grams by the 17.6 cc. pipette, etc. Such points are minor except to illustrate the difficulty encountered in preparing an introductory book which must give positive statements with little explanation, and the need for tolerance in interpreting every word and phrase with absolute exactness.

In these days when plant managers, advertising specialists, economists, etc., enter the dairy industry without an intimate knowledge of the industry, this book ought to make a special appeal to them. The information would be as valuable and available to them as it is to the college student.

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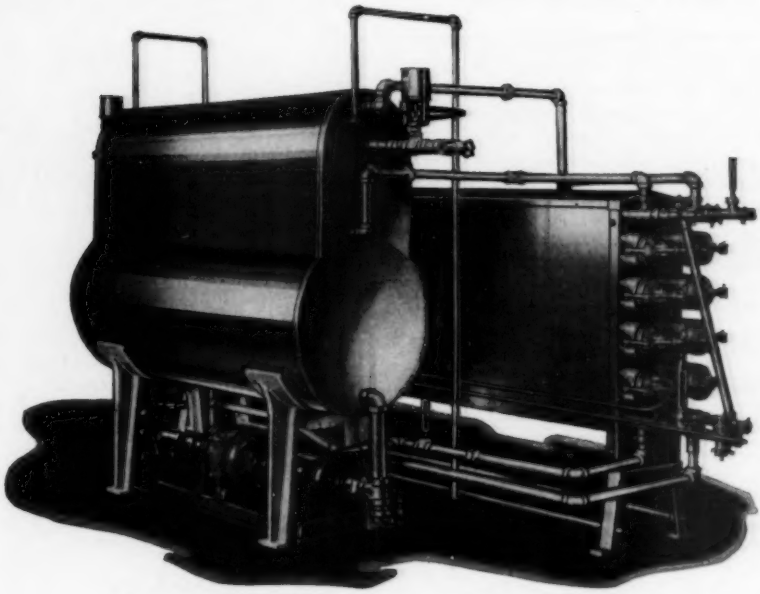

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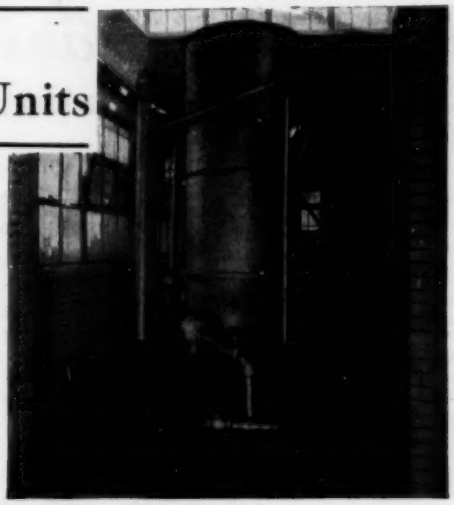

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Editorial comment in April 1929 issue of "Certified Milk", p. 88

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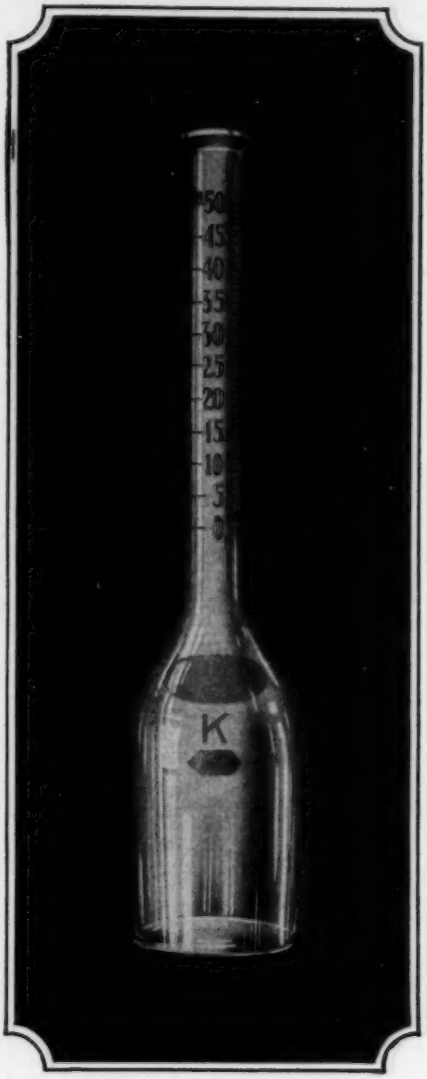

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